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# Carbon and Nitrogen Isotopic Investigations of the Late Pleistocene Paleoecology of Eastern Beringia, Yukon Territory, Using Soils, Plants and Rodent Bones

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A thesis submitted in partial fulfillment of the requirements for the degree in Doctor of Philosophy

# CARBON AND NITROGEN ISOTOPIC INVESTIGATIONS OF THE LATE PLEISTOCENE PALEOECOLOGY OF EASTERN BERINGIA, YUKON TERRITORY, USING SOILS, PLANTS AND RODENT BONES

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# Abstract

During the late Pleistocene (130-12 ka), Beringia, a largely ice-free land located in the Mammoth Steppe Ecosystem, was home to a large grazing community of megafauna. Many of these animals, including the woolly mammoth, became extinct at the terminal Pleistocene. Assessment of the paleoenvironment, nutrient cycling and foraging ecology in Beringia should help to understand the role of climate change in their extirpation. Such information might also help to explain the curiously higher $\delta^{15}N$ of woolly mammoths relative to other coeval herbivores.

This study assessed eastern Beringian paleoecology using stable nitrogen (N) and carbon (C) isotopic analyses of late Pleistocene fossil and modern soils, plants and rodent bones from Yukon Territory. A principal goal was to determine whether a modern isotopic baseline for the grassland food web should be used to interpret the isotopic data for Beringian megafauna. The average $\delta^{13}C$ of fossil plants from fossil arctic ground squirrel nests is 1.6 ‰ higher than modern equivalents, mostly because of the Suess effect. Experiments using modern grasses suggest that the fossil plants were enriched in $^{15}N$ by microbially mediated decomposition. The pre-decomposition $\delta^{15}N$ of the fossil plants was determined by measuring the N isotopic spacing between modern arctic ground squirrel collagen and their diet, and applying this spacing to the fossil bones. The results show that the $\delta^{15}N$ of fossil plants was originally ~2.5 ‰ higher than their modern equivalents. This difference suggests a shift in N cycling between the late Pleistocene and present time. A controlled-growth study of the effects of progressive $CO_2$ enrichment – which occurred after the terminal Pleistocene – on subarctic nutrient cycling, demonstrates a pattern of decreasing $\delta^{13}C$ for mature grasses, although the change was not significant. The $\delta^{15}N$ response was nonlinear and not significant.

The collective results indicate that modern plant isotopic compositions for Yukon Territory are not appropriate baselines for interpreting the $\delta^{13}C$ and $\delta^{15}N$ of Pleistocene megafauna from eastern Beringia. In addition, decomposed plants, which are typically enriched in $^{15}N$, may have been important foods that were preferentially available to the woolly mammoth during cold seasons, and thus biased their $\delta^{15}N$ to higher values.

## Keywords

Beringia, Pleistocene, nitrogen, carbon, isotopes, soil, plant, bone, $CO_2$ enrichment.

# Epigraph

"Ice Age plants might be different from now
But the question we ask is: just how?
So in squirrel nests we peek
For the answers we seek
What isotopes still will allow."

– FJL–

# Dedication

This thesis is dedicated to my husband, Mahdi, and to my parents.

# Acknowledgments

Over the last four years I have had the privilege of meeting so many wonderful people, who have helped me in many ways.

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# Table of Contents

Abstract ..... ii

Epigraph ..... iv

Dedication ..... v

Acknowledgments ..... vi

Table of Contents ..... viii

List of Tables ..... xiii

List of Figures ..... xvi

List of Appendices ..... xxii

Chapter 1 ..... 1

1. Introduction and Overview ..... 1

1.1 Overview ..... 1

1.2 Carbon and Nitrogen Isotopic Analyses in the Study of Terrestrial Ecosystems: A Brief Introduction ..... 2

1.2.1 Stable Isotopes ..... 2

1.2.2 Nitrogen Cycle and Stable Nitrogen Isotopic Variations in Terrestrial Ecosystems ..... 3

1.2.3 Carbon Cycle and Stable C Isotopic Variations in Terrestrial Ecosystems 7

1.2.4 Stable Nitrogen and Carbon Isotopic Variations in Food Webs ..... 7

1.3 Setting: Mammoth Steppe Ecosystem and Beringia ..... 9

1.3.1 Modern Study Areas: Kluane Lake and Whitehorse ..... 11

1.3.2 Klondike Goldfields of West-central Yukon Territory ..... 12

1.4 Main Questions and Organization of the Dissertation ..... 13

1.5 References ..... 16

Chapter 2 ..... 29

2 Nitrogen and Carbon Isotopic Dynamics of Subarctic Soils and Plants in Southern Yukon Territory: Implication for Paleoecological and Paleodietary Studies ..... 29

2.1 Introduction ..... 29
2.1.1 Study Area: Kluane Lake and Whitehorse, Yukon ..... 31
2.1.2 Carbon Isotopic Composition of Soil and Terrestrial Plants ..... 37
2.1.3 Nitrogen Isotopic Composition of Soil and Terrestrial Plants ..... 42
2.2 Materials and Methods ..... 50
2.2.1 Sample Collection and Preparation ..... 50
2.2.2 Elemental Analysis ..... 52
2.2.3 Stable Isotope Analyses ..... 53
2.2.4 Statistical Analysis ..... 55
2.3 Results ..... 55
2.3.1 Soils ..... 55
2.3.2 Plants ..... 62
2.4 Discussion ..... 73
2.4.1 Soils of Kluane Lake Area ..... 73
2.4.2 Plant C and N Isotopic and Elemental Compositions ..... 78
2.4.3 Intra-plant Variations ..... 85
2.5 Conclusion ..... 86
2.6 References ..... 87
Chapter 3 ..... 106
3 The Eastern Beringian Nitrogen Cycle before and after the Terminal Pleistocene ... 106
3.1 Introduction ..... 106
3.1.1 Terrestrial N Cycling and $\delta^{15}$N of Soils and Plants ..... 108
3.1.2 Plants and Soils $\delta^{13}$C ..... 110
3.1.3 N, C and O Isotopic Compositions of Bone ..... 111
3.1.4 Decomposition of Plant Materials ..... 114
3.2 Study Area: Placer Goldmines of West-central Yukon Territory ..... 119

3.3 Materials and Methods ..... 122
3.3.1 Design of Decomposition Experiment ..... 122
3.3.2 Sample Collection ..... 123
3.3.3 Sample Preparation ..... 126
3.3.4 Elemental Analysis ..... 129
3.3.5 Stable Isotope Analyses ..... 130
3.3.6 Microscopic Analysis ..... 132
3.3.7 Statistical Analysis ..... 132
3.4 Results ..... 132
3.4.1 Paleosols ..... 132
3.4.2 Fossil Plants ..... 136
3.4.3 Radiocarbon Dates ..... 139
3.4.4 Water Isotopic Composition ..... 139
3.4.5 Modern Plants ..... 139
3.4.6 Fossil and Modern Bone Isotopic Compositions ..... 143
3.4.7 $^{13}C$ and $^{15}N$ Enrichment of Ground Squirrel Bone Collagen relative to Plants in Modern Study Areas ..... 150
3.4.8 Modern Plant Decomposition Experiment ..... 151
3.5 Discussion ..... 163
3.5.1 Modern Plant Decomposition Experiment ..... 163
3.5.2 Paleosols ..... 172
3.5.3 Plant Macrofossils ..... 173
3.5.4 Fossil and Modern Bone Collagen and Bioapatite Structural Carbonate 173
3.5.5 Late Pleistocene Beringia and Possible Modern Comparisons ..... 176
3.5.6 Modern and Ancient C and N Isotopic Baselines ..... 177
3.6 Conclusion ..... 184

3.7 References ..... 185

Chapter 4 ..... 209

4 Response of Subarctic Terrestrial Carbon and Nitrogen Isotopic Baselines to $CO_2$ Enrichment: Controlled Experiments ..... 209

4.1 Introduction ..... 209

4.1.1 Plant C Content and $\delta^{13}C$ Variations with Change in $pCO_2$ ..... 210

4.1.2 Plant N and $\delta^{15}N$ Variations with Change in $pCO_2$ ..... 213

4.2 Materials and Methods ..... 214

4.2.1 Design of Experiments and Growth Conditions ..... 214

4.2.2 Sample Collection ..... 216

4.2.3 Sample Preparation ..... 217

4.2.4 Elemental Analysis ..... 217

4.2.5 Isotopic Analysis ..... 217

4.2.6 Statistical Analysis ..... 218

4.3 Results ..... 218

4.3.1 Carbon Isotopic Composition of $CO_2$ in Growth-Chambers ..... 218

4.3.2 Carbon Isotopic Results ..... 219

4.3.3 Nitrogen Isotopic Results ..... 230

4.3.4 Carbon and Nitrogen Elemental Results ..... 235

4.4 Discussion ..... 248

4.4.1 Intra-plant Variations in $\delta^{13}C$ and $\delta^{15}N$ within $CO_2$ Treatments ..... 248

4.4.2 Intra-plant Variations in C and N Contents ..... 249

4.4.3 Carbon Elemental and Isotopic Variations with $CO_2$ Enrichment ..... 249

4.4.4 Nitrogen Elemental and Isotopic Variations with $CO_2$ Enrichment ..... 252

4.5 Conclusion ..... 255

4.5.1 Implication in Paleoecology ..... 255

4.5.2 Implication in Designing Controlled Growth Experiments ..... 256
4.6 References ..... 256
Chapter 5 ..... 268
5 Conclusions ..... 268
5.1 Summary and Future Directions ..... 268
5.2 Why did the Woolly Mammoth have higher $\delta^{15}$N than coeval herbivores? ..... 271
5.2.1 Different Dietary Niches ..... 272
5.2.2 Different Microhabitats ..... 275
5.2.3 A Change in N Isotopic Baseline ..... 276
5.2.4 Synopsis ..... 276
5.3 References ..... 277
Appendices ..... 283
Curriculum Vitae ..... 340

# List of Tables

Table 2-1: Climatic data for Kluane Lake and Whitehorse study areas. ..... 34

Table 2-2: N isotopic fractionation for different biochemical reactions in the N cycle (from Robinson, 2001 and Houlton and Bai, 2009)..... 45

Table 2-3: Environmental data for sampling sites and number of soils and plants sampled.. 57

Table 2-4: Soil characteristics..... 58

Table 2-5: Soil mineralogy. ..... 59

Table 2-6: Isotopic composition of soil TN and OC..... 60

Table 2-7: One-way ANOVA *p*-value results for isotopic differences among all plant tissues. ..... 67

Table 2-8: One-way ANOVA *p*-value results for differences in foliar $\delta^{13}$C and $\delta^{15}$N between different sites..... 68

Table 2-9: One-way ANOVA *p*-value results for differences in C and N contents between different plant tissues. ..... 71

Table 2-10: Independent-samples t-test results for differences in foliar C and N contents between plant functional groups. ..... 71

Table 3-1: General data for sampling sites. ..... 134

Table 3-2: Paleosol characteristics..... 135

Table 3-3: Paleosol mineralogy. ..... 135

Table 3-4: Isotopic composition of soil TN and OC..... 136

Table 3-5: C and N isotopic and elemental compositions of bulk plants recovered from fossil nests..... 140

Table 3-6: $\delta^{18}O$ and $\delta^{2}H$ of water. ..... 143

Table 3-7: Fossil and modern bones collagen $\delta^{13}C$, $\delta^{15}N$, C and N contents (wt. %) and yield, and structural carbonate $\delta^{18}O$ and $\delta^{13}C$..... 146

Table 3-8: CI and C/P values for fossil and modern bones (calculated using FTIR spectra). ..... 148

Table 3-9: The $\delta^{18}O$ of structural carbonate and drinking water for bones. ..... 149

Table 3-10: Weather data for London, ON, Canada. ..... 152

Table 3-11: Average $\delta^{13}C$ and $\delta^{15}N$ of plant detritus during decomposition. ..... 154

Table 3-12: *p*-value results of repeated measure ANOVA (Greenhouse-Geisser) test for time-dependent effects on $\delta^{13}C$ and $\delta^{15}N$ of plant detritus. ..... 155

Table 3-13: *p*-value results of Benferroni *post hoc* test for differences in average $\delta^{13}C$ between Day 1 and days 164, 253 and 317 for samples showing significant time-dependent effect after the repeated measure ANOVA (Greenhouse-Geisser) test. ..... 156

Table 3-14: *p*-value results of Benferroni *post hoc* test for differences in average $\delta^{15}N$ between Day 1 and days 164, 253 and 317 for samples showing significant time effect after the repeated measure ANOVA (Greenhouse-Geisser) test. ..... 156

Table 3-15: Average C and N contents and atomic C/N of plant detritus during decomposition. ..... 161

Table 4-1: Average growth-chamber $\delta^{13}C_{CO2}$. ..... 219

Table 4-2: Average $\delta^{13}C$ and $\delta^{15}N$ of plant parts and rhizosphere soil for different $CO_2$ treatments ..... 221

Table 4-3: Average $\Delta^{13}C_{RC\text{-}L}$ and $\Delta^{13}C_{FR\text{-}L}$ for each $CO_2$ treatment. ..... 223

Table 4-4: One-way ANOVA *p*-value results for $\Delta^{13}C$ among plant tissues for each $CO_2$ treatment. ..... 224

Table 4-5: One-way ANOVA *p*-value results for differences in $\delta^{13}C$ and $\delta^{15}N$ of different plant parts between $CO_2$ treatment. ..... 227

Table 4-6: Average $\Delta^{15}N_{RC\text{-}L}$ and $\Delta^{15}N_{FR\text{-}L}$ for each $CO_2$ treatment. ..... 231

Table 4-7: One-way ANOVA *p*-value results for $\delta^{15}N$ differences among plant tissues for each $CO_2$ treatment. ..... 232

Table 4-8: Average C and N contents of plant parts and rhizosphere soil for different $CO_2$ treatments. ..... 236

Table 4-9: Average difference ($\Delta$) in C and N contents between tissues types for each $CO_2$ treatment. ..... 238

Table 4-10: One-way ANOVA *p*-value results for differences in C contents among plant tissues for each $CO_2$ treatment. ..... 239

Table 4-11: One-way ANOVA *p*-value results for N contents among plant tissues for each $CO_2$ treatment..... 240

Table 4-12: One-way ANOVA results for differences in C and N contents of different plant parts between $CO_2$ treatments. ..... 244

Table 4-13: Average atomic C/N of different plant parts under different $CO_2$ treatments... 246

# List of Figures

Figure 1-1: N biogeochemical cycle in the soil-plant-consumer-atmosphere of terrestrial ecosystems; solid lines present main input and internal N reactions and dashed lines present main N loss reactions (adapted from Havlin et al., 2005) ..... 4

Figure 1-2: The maximum extent of Beringia during the late Pleistocene (adapted from http://www.thecraftycrow.net/crafts-around-the-world/). ..... 9

Figure 1-3: The $\delta^{15}N$ and $\delta^{13}C$ variations of bone collagen from Late Pleistocene mammoth steppe megaherbivores for: (a) entire ecosystem, and (b) Siberia, Alaska and Scladina (Adapted from Bocherens, 2003)..... 16

Figure 2-1: Location of study areas and sampling sites..... 33

Figure 2-2: Kluane Ranges, southwest Yukon Territory and Alaska. ..... 34

Figure 2-3: Dust storms in Slims River Delta, September 2012 (photograph credit: Fred Longstaffe)..... 35

Figure 2-4: Soil profiles at Kluane Lake (S13-6 and S13-8; photographic credit: Tessa Plint). See Figure 2-1 for site location..... 36

Figure 2-5: Vegetation cover of the eastern shoreline of Kluane Lake (photographic credit: Tessa Plint)..... 37

Figure 2-6: $C_3$ and $C_4$ photosynthetic pathways. ..... 38

Figure 2-7: A simple model of N cycling in the soil-plant-atmosphere system. ..... 43

Figure 2-8: Depth profiles of $\delta^{13}C_{OC}$ and $\delta^{15}N_{TN}$ at (a) sites S13-6 and (b) S13-8 (data points provide average result for soil interval sampled (vertical line)). ..... 61

Figure 2-9: Differences in $\delta^{13}C$ between other plant tissues and leaf ($\Delta^{13}C$). ..... 64

Figure 2-10: Differences in $\delta^{15}N$ between other plant tissues and leaf ($\Delta^{15}N$). ..... 65

Figure 2-11: Mean (± SD) $\delta^{13}C$ and $\delta^{15}N$ of all plant parts analyzed. ..... 66

Figure 2-12: Foliar $\delta^{15}N$ *vs.* $\delta^{13}C$ for all plant samples..... 67

Figure 2-13: Sites sampled in 2012 (≥ 5 plants) (photographic credits: Fred Longstaffe)..... 69

Figure 2-14: Sites sampled in 2013 (≥ 5 plants) (photographic credits: Tessa Plint)..... 70

Figure 2-15: Average N and C contents of plant parts according to sampling year. ..... 72

Figure 2-16: Average foliar and whole plant carbon and nitrogen contents of different plant functional groups. ..... 72

Figure 2-17: Foliar $\delta^{15}N$ *vs.* N (wt. %). ..... 73

Figure 2-18: (a) Areal view of the Slims River delta; (b) Slims River vegetation in August (photographic credits: Fred Longstaffe and Tessa Plint)..... 75

Figure 2-19: Comparison of topsoil and foliar C and N isotopic compositions. ..... 76

Figure 2-20: Site S13-10 (photographic credit: Tessa Plint). ..... 78

Figure 2-21: Nitrogen *vs.* carbon isotopic compositions of all plant parts. ..... 79

Figure 2-22: A simplified model to explain a positive correlation between plant $\delta^{15}N$ and $\delta^{13}C$. ..... 80

Figure 2-23a: A simplified model for the "openness" of the N cycle in ecosystems with high N availabilities. (a) N mineralization: Conversion of organic N to $NH_4^+$ (ε = 0-5 ‰); (b) Microbial assimilation: incorporation of $NH_4^+$ into microbial biomass (ε = 14-20 ‰). (c) $NH_3$ volatilization: conversion of $NH_4^+{}_{(aq)}$ to $NH_{3(g)}$ (ε = 40-60 ‰); (d) Nitrification: conversion of $NH_4^+$ to $NO_3^-$ (ε = 15-35 ‰); (e) Plant uptake and assimilation of $NH_4^+$ (ε = 9-18 ‰); (f) Plant uptake and assimilation of $NO_3^-$ (ε = 0-19 ‰); (g) $NO_3^-$ leaching (ε = 0-1 ‰); (h) Denitrification: conversion of $NO_3^-$ to $N_2O$, $N_2$ and $NO_2$ (ε = 28-33 ‰), (ε data from Robinson, 2001and Houlton and Bai, 2009)..... 82

Figure 2-23b: A simplified model for the “openness” of the N cycle in ecosystems with low N availabilities. (a) N mineralization: Conversion of organic N to $NH_4^+$ (ε = 0-5 ‰); (b) Microbial assimilation: incorporation of $NH_4^+$ into microbial biomass (ε = 14-20 ‰). (c) Nitrification: conversion of $NH_4^+$ to $NO_3^-$ (ε = 15-35 ‰); (d) Plant uptake and assimilation of $NH_4^+$ (ε = 9-18 ‰); (e) Plant uptake and assimilation of $NO_3^-$ (ε = 0-19 ‰), (ε data from Robinson, 2001and Houlton and Bai, 2009). ..... 83

Figure 3-1: N cycling in terrestrial ecosystems; solid lines indicate main input and internal N reactions and dashed lines indicate main N loss reactions (adapted from Havlin et al., 2005). ..... 109

Figure 3-2: Location of sampling sites (After Kotler and Burn, 2000). ..... 121

Figure 3-3: Decomposition experiment (a) before and (b) after outdoor placement. ..... 123

Figure 3-4: Arctic ground squirrel nest found in perennially frozen paleosol in Quartz Creek (photographic credit: Fred Longstaffe). ..... 125

Figure 3-5: Mining cuts at Quartz Creek (photographic credit: Fred Longstaffe). ..... 125

Figure 3-6: A sequence of paleosol layers at Quartz Creek (photographic credit: Fred Longstaffe). ..... 125

Figure 3-7: Typical plant macrofossils recovered from fossil nests. (a) *Carex* floret; (b) *Campylium stellatum* stem with leaves; (c) *Conioselinum cnidiifolium* carpel; (d) *Asteracea* achenese, pappus; (e) *Lepidium densiflorum*; (f) *Phlox hoodii* capsule; (g) *Taraxacum ceratophorum* achene; (h) *Pedicularis* sp. achene; (i, j) *Silene cf. taymirensis* capsule with seeds inside; (k) *Polemonium* capsule; (l, n) *Ranunculuc pensylvanicus-macounii* achene; (m) *Draba* spp. capsule; (o) *Plantago* cf. *canescens* capsule. ..... 137

Figure 3-8: Typical plant macrofossils recovered from fossil ground squirrel nests. (a) *Deschampsia caespitose* floret; (b) *Deschampsia caespitose* floret with stem; (c) *Carex albo niagra* floret and seed; (d) *Alopecurus* sp. Floret; (e) *Draba* sp. Silique; (f) *Ranunculus eschscholtzii-sulphureus* achene. ..... 138

Figure 3-9: Fossil bones recovered from fossil nests. ..... 144

Figure 3-10: Average $\delta^{13}C$ and $\delta^{15}N$ of modern plants and ground squirrel bone collagen from Kluane Lake and Whitehorse. ..... 150

Figure 3-11: Average $^{13}C$ and $^{15}N$ enrichment from bulk plant to ground squirrels bone collagen from Kluane Lake and Whitehorse. ..... 151

Figure 3-12: Variation in plant $\delta^{13}C$ during decomposition under (a) 'buried', and (b) 'not buried' conditions. ..... 157

Figure 3-13: Variation in plant $\delta^{15}N$ during decomposition under (a) 'buried', and (b) 'not buried' conditions. ..... 158

Figure 3-14: Variation in atomic C/N shifts during decomposition under (a) 'buried' and (b) 'not buried' conditions. ..... 160

Figure 3-15: Images of *E. trachycaulus and F. altaica*: (1) before decomposition, (2) after decomposition on soil surface, and (3) after decomposition in soil. ..... 165

Figure 3-16: SEM images of *F. altaica*: (1) before decomposition, (2) after decomposition on soil surface, and (3) after decomposition in soil. (Yellow arrows: fungal hypha)..... 166

Figure 3-17: Earthworms (red arrows) and their casts (yellow arrows) in vicinity of 'buried' plant detritus..... 167

Figure 3-18: Model for plant decay and main processes contributing to C and N isotopic enrichment of decomposing plant tissues: (1) leaching of $^{15}N$-depleted compounds, (2) C mineralization and release of $^{13}C$-depleted $CO_2$, and (3) microbial N assimilation and dissimilation, during which $^{15}N$-depleted $NH_3$ is released into soil and $^{15}N$-enriched $NH_4^+$ is left in microbial biomass (Dijkstra et al., 2008). ..... 170

Figure 3-19: Bivariat plot of (a) $\delta^{15}N$ *vs.* atomic C/N, and (b) $\delta^{15}N$ *vs.* C (wt. %) of decomposed plant tissues. ..... 171

Figure 3-20: Average $\delta^{15}N$ *vs.* $\delta^{13}C$ of fossil and modern samples. ..... 178

Figure 3-21: Average N *vs.* C contents of fossil and modern plants. ..... 179

Figure 3-22: SEM images of plant macrofossils: (a, b) decomposed surface, and (c, d) fungal hypha (yellow arrows) and bacteria cells (red arrows). ..... 180

Figure 3-23: Measured fossil plant $\delta^{13}C$ *vs.* fossil plant $\delta^{13}C$ estimated using fossil bone $\delta^{13}C$ and modern $\Delta^{13}C_{Col\text{-}bulk\ plant}$ = +4.7 ‰. ..... 180

Figure 3-24: Measured fossil plant $\delta^{15}N$ *vs.* fossil plant $\delta^{15}N$ estimated using fossil bone $\delta^{15}N$ and modern $\Delta^{15}N_{Col\text{-}bulk\ plant}$ = +2.0 ‰. ..... 181

Figure 3-25: $\delta^{15}N$ *vs.* $\delta^{13}C$ of plant macrofossils after correction using bone $\delta^{15}N$ and $\delta^{13}C$ and modern $\Delta^{15}N_{Col\text{-}bulk\ plant}$ and $\Delta^{13}C_{Col\text{-}bulk\ plant}$. The isotopic results for fossil bone collagen are also shown. Squares: plants; Circles: bone collagen. Samples from the same nest are shown in the same color. ..... 181

Figure 3-26: $\delta^{15}N$ *vs.* $\delta^{13}C$ of fossil and modern plants and bone collagen (average $\delta^{15}N$ and $\delta^{13}C$ for fossil plants are calculated). ..... 182

Figure 4-1: Change in $\delta^{13}C$ of seedlings with $CO_2$ enrichment (ambient, 800 and 1000 ppm). Averages ± SD (n = 6-9) are illustrated. ..... 226

Figure 4-2: Change in $\delta^{13}C$ of mature plants with $CO_2$ enrichment (ambient, 800 and 1000 ppm). Averages ± SD (n = 6-9) are illustrated. ..... 229

Figure 4-3: Change in $\delta^{15}N$ of seedlings and rhizosphere soil with $CO_2$ enrichment (ambient, 800 and 1000 ppm). Averages ± SD (n = 6-9) are illustrated. ..... 233

Figure 4-4: Change in $\delta^{15}N$ of mature plants with $CO_2$ enrichment (ambient, 800 and 1000 ppm). Averages ± SD (n = 6-9) are illustrated. ..... 234

Figure 4-5: Change in C and N contents of seedling plant parts and rhizosphere soil with $CO_2$ enrichment (ambient, 800 and 1000 ppm): (1) *P. glauca*, (2) *E. macrourus*, and (3) *B. pumpellianus*. ..... 242

Figure 4-6: Change in C and N contents of mature plant parts and rhizosphere soil with $CO_2$ enrichment (ambient, 800 and 1000 ppm): (1) *P. glauca*, (2) *B. pumpellianus*, and (3) *F. altaica*. ..... 243

Figure 4-7: Change in atomic C/N of plant parts with $CO_2$ enrichment (ambient, 800 and 1000 ppm). ..... 247

# List of Appendices

Appendix A: Accepted $\delta$ values and elemental concentrations for international and internal standards used in this study..... 283

Appendix B: Corrections to elemental composition data. ..... 285

Appendix C: Accuracy and precision for standards, exclusive of those used to generate calibration curves. ..... 286

Appendix D: $\delta^{13}C$ and $\delta^{15}N$ of all plant parts sampled in 2012 and 2013. ..... 290

Appendix E: Carbon and Nitrogen contents of all plants according to plant parts and year of sampling. ..... 295

Appendix F: Foliar atomic C/N of modern plants according to year of sampling. ..... 300

Appendix G: Accuracy and precision for standards associated with data presented in Chapter 3. Mean isotopic and elemental compositions are not reported for standards used to generate calibration curves. ..... 303

Appendix H: (H.1) $\delta^{13}C$ and $\delta^{15}N$ of all replicates in the decomposition experiment..... 307

Appendix I: Effects of Grazing and Dung Fertilization on Plant Nitrogen Isotopic Compositions: Controlled Experiments ..... 311

Appendix J: Accuracy and precision for standards associated with data presented in Chapter 4. Mean isotopic and elemental compositions are not reported for standards used to generate calibration curves. ..... 324

Appendix K: $\delta^{13}C$ (K.1) and $\delta^{15}N$ (K.2) of different plant parts and species examined for each $CO_2$ treatment..... 328

Appendix L: C (L.1) and N (L.2) contents of different plant parts and species examined in each treatment. ..... 334

# Chapter 1

## 1. Introduction and Overview

### 1.1 Overview

During the late Pleistocene (130-10 ka) (Blinnikov et al., 2011), an unglaciated portion of the northwest of Canada, Alaska and Siberia, named Beringia, was home to a large grazing community of megafauna (animals weighing > 44 kg). The majority of these megafauna went extinct during the terminal Pleistocene to Holocene transition (12,500-10,000 $^{14}$C a BP (Anderson and Lozhkin, 2015)). Megafauna's extinctions in western North America were dramatic, claiming 35 genera, most of them large bodied (Faith and Surovell, 2009). Multiple hypotheses have been proposed for this extinction event, including climate change (Mann et al., 2013), habitat change (Gill et al., 2009), the 'overkill' hypothesis that points to prehistoric humans as efficient predators (Surovell et al., 2005), or a combination of all of these possibilities (MacDonald et al., 2012).

Beringia is a unique terrestrial model for the study of paleoclimate and the environmental responses of high Arctic and subarctic ecosystems to the frequent, high amplitude fluctuations in climate during the late Pleistocene. It is a great repository of different terrestrial fossils that record these responses over a wide range of time intervals. Much effort has been devoted to understanding this unique ecosystem, which has no exact modern analogue (Gill et al., 2009; Höfle et al., 2000; Williams et al., 2001). Investigations into its basic structure have examined soil (Höfle et al., 2000; Hofle and Ping, 1996; Sanborn et al., 2006), vegetation (Swanson, 2006; Zazula et al., 2003, 2006b), animal communities and their dynamics through time (Guthrie, 1968, 1982, 1990; MacDonald et al., 2012; Mann et al., 2013; Pushkina et al., 2014), climate (Elias, 2000; Fritz et al., 2012a; Jones et al., 2014; Kuzmina et al., 2008), and the relative contributions of humans and climate to megafaunal extinction during the late Pleistocene to Holocene transition (Koch and Barnosky, 2006). These works have involved a wide range of methodologies, including radiocarbon dating, stable isotope analyses, paleontology, sedimentology, tephrochronology and genetic studies of different proxies

such as macro- and micro-fossils of vertebrate and invertebrate fossils, plant macrofossils and their phytoliths, pollen, macro- and micro-tephras, and paleosols.

Despite these efforts, some important aspects of this ecosystem have remained poorly understood, especially:

1. Nutrient cycling in this ecosystem during the late Pleistocene, which was important in maintaining the productive ecosystem needed to support the megafaunal population,
2. Possible changes in nutrient cycling in this ecosystem associated with climatic and environmental changes during the late Pleistocene to Holocene transition, and
3. Diet, feeding habits and foraging ecology of the vanished megafauna.

This dissertation endeavors to address these aspects in eastern Beringia (Yukon Territory, Canada; described more fully in section 1.4) after first providing brief background information concerning (i) stable isotopes, (ii) nitrogen (N) and carbon (C) cycling in terrestrial ecosystems, (iii) N and C isotopic behavior in vegetation and food webs, and (iv) the Mammoth Steppe Ecosystem, Beringia and the specific study areas in Yukon Territory.

## 1.2 Carbon and Nitrogen Isotopic Analyses in the Study of Terrestrial Ecosystems: A Brief Introduction

### 1.2.1 Stable Isotopes

Stable isotopes are different species of the same chemical element with the same number of electrons and protons, but different numbers of neutrons. Stable isotopes do not undergo decay like radioactive ones. A good example for stable and radioactive isotopes of an element is provided by the isotopes of C: $^{14}C$, $^{13}C$ and $^{12}C$. Its stable isotopes are $^{13}C$ and $^{12}C$, while $^{14}C$ is radioactive and produced in the upper atmosphere through reactions in which $^{14}N$ absorbs thermal neutrons produced from cosmic rays.

The heavier (i.e., higher mass) stable isotope of an element is normally less abundant than the lighter one. All stable forms of an element behave similarly in chemical reactions due to their identical number of protons and electrons, but have different physical properties due to their different atomic mass. These differences produce varying ratios of heavier to lighter stable isotopes among different compounds in nature. These ratios can provide useful information about the source, the fate, reaction mechanisms, reaction environment and – more generally – the history of a compound (Schoeller, 1999).

Because the difference in absolute abundances of various stable isotopes of an element distributed among substrates and products of different biogeochemical reactions in nature is extremely small, it is amplified by a factor of 1000 (parts per thousand, ‰) and reported relative to an international standard using the $\delta$-notation:

Equation 1.1 $$\delta^{H}E = [R_{Sa} / R_{Std}] - 1$$

where E represents an element (e.g. C, N, O), H represents the heavy isotope of that element, and $R_{Sa}$ and $R_{Std}$ are the ratio of heavy to light isotope in sample and standard, respectively. The stable isotopes of hydrogen ($^{2}H/^{1}H$), carbon ($^{13}C/^{12}C$), nitrogen ($^{15}N/^{14}N$) and oxygen ($^{18}O/^{16}O$) are the focus of analytical investigation in this dissertation.

### 1.2.2 Nitrogen Cycle and Stable Nitrogen Isotopic Variations in Terrestrial Ecosystems

Among major nutrients for plants (N, P, K, S, Ca, Mg and Mn), N is unique because it is the only one originating mainly from the atmosphere (the rest are derived from minerals). Despite the large reservoir of N in the atmosphere ($N_2$), it is rarely accumulated in bio-available forms for plants in soils, and hence needs to be replenished continuously through N mineralization. Not surprisingly, N deficits limit the productivity of most terrestrial ecosystems (Lebauer and Treseder, 2008). In the biogeochemical cycle of N in terrestrial ecosystems (Fig. 1-1), N enters soil from either decomposition of organic matter or biotic and abiotic N deposition from the atmosphere. Nitrogen then undergoes a

series of biochemical reactions that characterize active N cycling within the soil-plant-consumer-atmosphere loop.

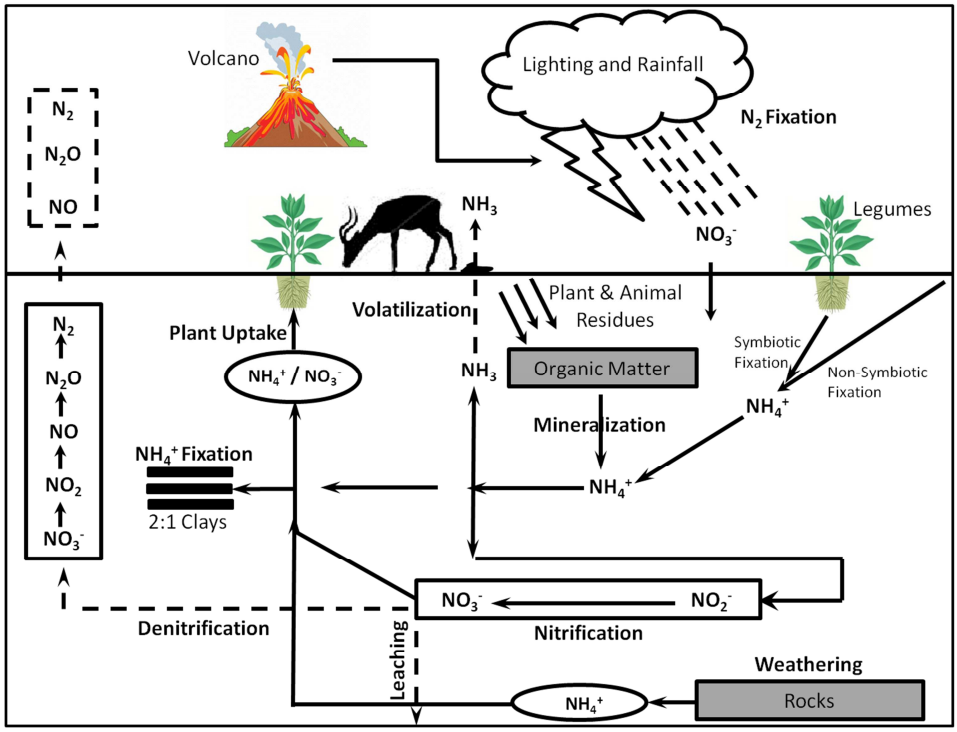


**Figure 1-1: N biogeochemical cycle in the soil-plant-consumer-atmosphere of terrestrial ecosystems; solid lines present main input and internal N reactions and dashed lines present main N loss reactions (adapted from Havlin et al., 2005)**

The importance of each process in the N cycle is ecosystem-specific and is highly affected by climatic and environmental factors. The ratio of N loss from the system by various processes (denitrification, volatilization and leaching) to internal N cycling (nitrification, plant uptake and mineralization) determines the openness of the N cycle (Reichmann et al., 2013). More loss through emissions of N-containing gases into the atmosphere relative to input of N and its cycling between plants and microbes provides evidence of a more open N cycle. Ecosystems with a higher availability of N are known to have more open N cycle than more N limited ecosystems (Martinelli et al., 1999). Knowledge of the balance between N inputs and losses in each ecosystem and the magnitude of the various processes affecting N is important when addressing questions regarding ecosystem productivity, pollution and floral and faunal extinctions or survival.

Nitrogen isotopic measurements are a valuable tool for studying the N cycle in different ecosystems (Martinelli et al., 1999; Nagel et al., 2013). Plant $\delta^{15}N$ can reflect the nature of N cycling in terrestrial ecosystems, with higher $\delta^{15}N$ indicating greater N availability and a more open N cycle (Hietz et al., 2011; Hogberg, 1997; Pardo et al., 2006). Higher N availability in soils means extra N for $^{15}N$-enriching processes (denitrification and volatilization) (Hogberg, 1997; Robinson, 2001). In such systems plants acquire N from a $^{15}N$-enriched pool and thus acquire higher $\delta^{15}N$. The magnitude and type of N loss processes can vary considerably depending on the ecosystem and its soil conditions (particle size distribution, temperature, pH, water availability) (Havlin et al., 2005).

Plant N isotopic signals are a consequence of source N isotopic composition and the factors affecting it. Most plants acquire N primarily from soils. Inorganic – nitrate and ammonium – and organic – simple amino acids, peptides and proteins – pools of N in soil have isotopic compositions controlled by the isotopic fractionations associated with the various biochemical reactions involved (Makarov, 2009). Bulk soil $\delta^{15}N$ is not necessarily a good measure of bioavailable N because the soil might be dominated by N sources not taken up by plants (Hogberg, 1997). Gradients in $\delta^{15}N$ for different N pools with soil depth add to this complexity, given the variation among plants in the morphology and depth distribution of their roots (Makarov, 2009). Increasing bulk soil $\delta^{15}N$ with depth has been reported in many studies, but no general depth-dependent isotopic pattern for inorganic forms has emerged (Hogberg, 1997; Koba et al., 1998; Makarov et al., 2008; Martinelli et al., 1999; Nadelhoffer and Fry, 1988).

Symbiotic association between (i) plants and mycorrhizal fungi, and (ii) $N_2$ fixing bacteria (rhizobia) and legumes, as well as herbivory activities (dung fertilization, trampling and grazing), are also factors that could affect the N isotopic signals of plants. Non-mycorrhizal plants tend to be enriched in $^{15}N$ relative to arbuscular mycorrhizal, ectomycorrhizal and ericoidmycorrhizal plants (Craine et al., 2009; Michelsen et al., 1998; Michelsen et al., 1996). The lower $\delta^{15}N$ of mycorrhizal plants has been attributed to discrimination against $^{15}N$ during transfer of N from fungi to host plants (Schimidt and Stewart, 1997) or to preferential transfer of $^{15}N$-depleted N compounds (e.g. glutamine)

produced during enzymatic reactions within fungi to its plant partner (Hobbie and Hobbie, 2008; Hobbie et al., 1999). Rhizobia bacteria can likewise affect the N isotopic signal of bioavailable N by their association with legumes to fix atmospheric $N_2$. This symbiosis provides these plants with N sources having $\delta^{15}N$ close to that of atmospheric $N_2$ (0 ‰) (Kohl and Shearer, 1980; Mariotti, 1983). An important role for herbivores in increasing soil and plant $\delta^{15}N$ and N contents, and accelerating N cycling in ecosystems, has been suggested in many studies (Coetsee et al., 2010; Frank and Evans, 1997; Frank et al., 2000; McNaughton et al., 1988; Molvar et al., 1993; Pastor et al., 2006; Ruess and McNaughton, 1987). Among activities associated with herbivory, perhaps most important is the large amount of N returned to soil through dung and urine deposition (Haynes and Williams, 1993), which increases the N contents of the ecosystem in a form that is more readily available to plants (Augustine and Frank, 2001; Augustine and McNaughton, 2007; Frank et al., 2000; Li et al., 2010; McKendrick et al., 1980; McNaughton et al., 1988; Wal et al., 2004).

Among climatic factors, mean annual temperature (MAT) and mean annual precipitation (MAP) appear to significantly affect the $\delta^{15}N$ of soils and plants (Amundson, 2003; Austin and Vitousek, 1998; Craine et al., 2009; Ma et al., 2012). A systematic local and global decrease in soil and plant $\delta^{15}N$ with increasing MAP and decreasing MAT has been observed by Amundson et al. (2003) and Craine et al. (2009) for ecosystems with MAT $\geq$ –0.5°C. The relationship could be related to a change in: (i) the nature of N cycling in soil and plants, (ii) the rate of soil N transformations, (iii) dependency on mycorrhizal fungi (Craine et al., 2009), and/or (iv) soil organic C and clay and silt contents (Craine et al., 2015) with MAT and MAP.

In short, N cycling and the resulting soil and plant $\delta^{15}N$ in an ecosystem are sensitive to a range of climatic and environmental factors, which in turn can be tracked along trophic levels from primary producers to consumers (animals and humans) (Schwarcz, 1999; Szpak et al., 2010). These relationships have important implications for studies of paleodiet and paleoecology, as is discussed in later parts of this dissertation.

### 1.2.3 Carbon Cycle and Stable C Isotopic Variations in Terrestrial Ecosystems

The main source of carbon in plant photosynthesis is atmospheric $CO_2$, which is captured through the stomata of leaves. These stomata let $CO_2$ in and $H_2O$ out. Therefore, carbon gain and water loss are associated. When $CO_2$ enters the leaf, it can be dissolved in leaf water and becomes available for the enzymatic reactions of photosynthesis. Two main photosynthetic mechanisms of plants, $C_3$ and $C_4$, show distinct enzymatic fixation of $CO_2$, which causes different discrimination against $^{13}C$ that results in different $\delta^{13}C$ for plant tissues. The modern mean $\delta^{13}C$ for $C_3$ and $C_4$ plants are –27 ‰ and –13 ‰, respectively (Tieszen, 1991).

Environmental factors also affect plant $\delta^{13}C$, thus producing a broad range of isotopic compositions within each group: –38 to –22 ‰ for $C_3$ and –21 to –9 ‰ for $C_4$ plants (Tieszen, 1991). Important factors influencing $^{13}C$ discrimination include assimilation of respired $CO_2$, water availability, light intensity and $CO_2$ partial pressure ($pCO_2$); these parameters affect either enzymatic reactions or stomatal conductance (Tieszen, 1991). Plants growing under dense canopy or on the forest floor tend to have more negative $\delta^{13}C$ than plants from open environments, in response to re-assimilation of respired $CO_2$, which is highly depleted of $^{13}C$ relative to atmospheric $CO_2$ (Pond et al., 2014; Tieszen, 1991). A change in $pCO_2$ can also modify plant $\delta^{13}C$ by changing $^{13}C$ discrimination ($\Delta^{13}C$), which can vary as a function of internal leaf $pCO_2$, plant photosynthesis capacity, and the balance between these two steps (Tieszen, 1991). A decrease in plant $\delta^{13}C$ with an increase in $\Delta^{13}C$ has been reported in response to $CO_2$ enrichment (Polley et al., 1993; Van de Water et al., 1994), but this response is not universal (Arneth et al., 2002; Beerling, 1996; Beerling and Chaloner, 1992; Penuelas and Azcon-Bieto, 1992; Woodward, 1993).

### 1.2.4 Stable Nitrogen and Carbon Isotopic Variations in Food Webs

Stable isotope ratios are powerful tools for studying food webs and tracking the flow of energy and materials through trophic levels (Casey and Post, 2011). The $\delta^{13}C$ and $\delta^{15}N$ of

any organism reflect a combination of the isotopic composition of its diet and the isotopic fractionations during tissue formation (Casey and Post, 2011). It follows that the isotopic signatures of plants – and associated environmental information – should be traceable through the food chain in the tissue isotopic compositions of consumers. Such an approach should allow reconstruction of the foraging ecology and habitat of animals.

Animal tissues are typically enriched in $^{15}N$ relative to their diet by about +3 to +4 ‰ per trophic level, although some variations exist (Ambrose, 1991; Ambrose, 2000; Hilderbrand et al., 1996; Hobson, 1992). This isotopic separation is useful for approximating the trophic position of consumers with unknown foraging ecology and trophic position (Bocherens et al., 2011; Richards et al., 2008). The trophic enrichment of $^{13}C$ between whole consumer body and whole diet is typically small (< +2 ‰), but varies depending on tissue (Miller et al., 2008). The $\Delta^{13}C$ between bone collagen, which reflects mainly dietary protein (Ambrose and Norr, 1993), and whole diet is ~+5 ‰ (Van Der Merwe, 1982). This fractionation can vary among large and small mammals, with large wild mammals showing larger fractionation than small ones (Ambrose and Norr, 1993). The $^{13}C$ fractionation is even larger (c. +10 to +12 ‰) between whole diet and structural carbonate in bioapatite; the latter mainly reflects $\delta^{13}C$ signature of total C of diet (DeNiro and Epstein, 1978; Sullivan and Krueger, 1981; Thorp and Van Der Merwe, 1987). This difference in $\Delta^{13}C$ between whole diet and different tissues results from differences in tissue turnover time, molecular compositions and fractionation associated with different C routings through which different tissues are constructed from different constituents of source C (Lee-Thorp et al., 1989). Because the $\delta^{13}C$ of both bone collagen and structural carbonate is ultimately controlled by diet, the $\Delta^{13}C$ between these two tissues in an animal can be used to identify herbivory (7 ± 1.5 ‰), carnivory (4 ± 1 ‰) and omnivory in the consumer (Crowley et al. 2010; Lee-Thorp et al. 1989; Sullivan and Krueger, 1981).

## 1.3 Setting: Mammoth Steppe Ecosystem and Beringia

During the late Pleistocene, particularly the Last Glacial Maximum (LGM) (~23-15k $^{14}$C a BP, peaking at 18k $^{14}$C a BP (Clark et al., 2009; Fox-Dobbs et al., 2008; Mix et al., 2001)), steppe tundra was the most extensive biome on Earth (Zimov et al., 2012). In the northern hemisphere this biome, which is named Mammoth Steppe Ecosystem, included large portions of middle and southern Europe, northern Asia (including Siberia), and Alaska and Yukon in North America (Guthrie, 1990) (Fig. 1-2). Beringia, a major component of this biome, was a largely ice-free land that extended from the northwest of Canada (Mackenzie River) to the Kolyma River in northeastern Siberia (Höfle et al., 2000). It remained unglaciated during Quaternary glacial periods when global sea level was lower than today. Beringia was likely a refugium for a biome dominated by relatively nutrient-rich steppe vegetation and a diverse community of megaherbivores and megacarnivores (Bombin and Muehlenbachs, 1985; Guthrie, 1990). Beringia also served as a land bridge between North America and Eastern Siberia during this glacially induced period of lowered sea level, facilitating movement of plants, animals and humans (Guthrie, 1982; Oechel et al., 2014; Pushkina et al., 2014).

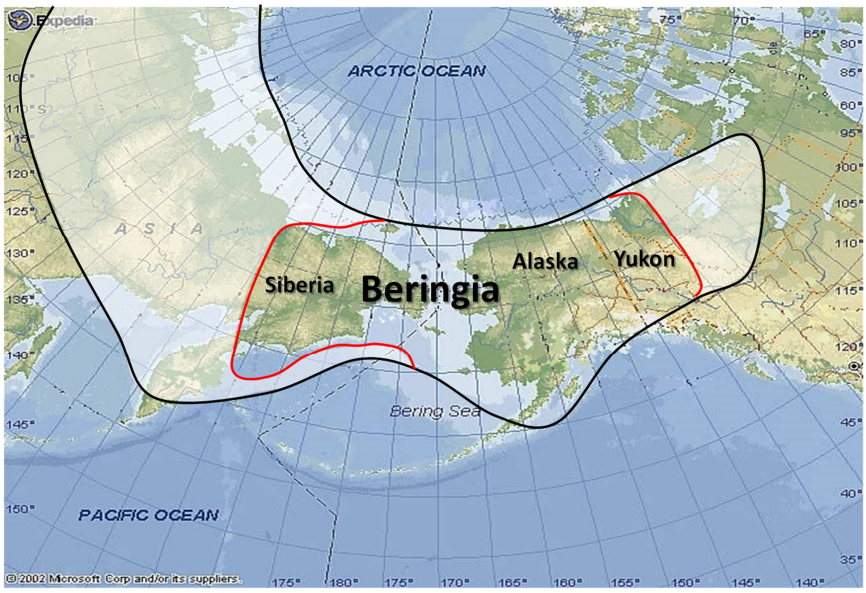


**Figure 1-2: The maximum extent of Beringia during the late Pleistocene (adapted from http://www.thecraftycrow.net/crafts-around-the-world/).**

Beringia was affected by frequent, high amplitude glacial cycles separated by short, warm interglacial periods during the late Pleistocene. Hence, Beringian sediments have great potential to record late Pleistocene Arctic and subarctic responses to these climatic fluctuations through preservation of macro- and micro-fossils. Given that Arctic and subarctic ecosystems are among the most sensitive regions to climate change, the study of past climate change in Beringia should provide better insight concerning responses to future climate change.

Late Pleistocene Beringia was a heterogeneous, ecological mosaic of vegetation communities, and regional microhabitats governed by local physical factors, including topography, topographic aspect, soil moisture, altitude, drainage, and loess deposition (Rainville et al., 2013; Sabat et al., 2012; Zazula et al., 2003, 2006a). Steppe bison, woolly mammoth, horse, camel and saiga were the most common grazing fauna of this region (Guthrie, 1968; Schweger, 1997). The steppe-tundra vegetation was dominated by grasses (*Poacea*), sedge (*Cyperaceae*), and sage (*Artemisia*) (Schweger, 1997; Zazula et al., 2002, 2006a). During Quaternary glacial episodes, in particular LGM, Beringia was located downwind of major glaciated zones, and hence characterized by intense eolian activities and accumulation of massive loess deposits (Fitzsimmons and Hambach, 2013; Guthrie, 1982; Thomas et al., 2014). It has been hypothesized that the thick layer of loess sediments was a key factor in maintaining the productivity of this ecosystem during the late Pleistocene (Guthrie, 1990; He et al., 2014; Laxton et al., 1996; Schweger, 1997). If so, there is no exact modern analogue against which Beringia can be compared (Höfle et al., 2000; Hofle and Ping, 1996; Schweger, 1997). The abundance and variety of Pleistocene mammalian fossils recovered from Beringian loess deposits portrays a specific vegetation mosaic that could support a nutritious diet for a great diversity of mammals with gigantic bodies (Guthrie, 1982; Jones et al., 2014), in contrast to the sparse herb tundra present today in Arctic ecosystems (Zazula et al., 2002).

At the time of the late Pleistocene to Holocene transition (12,500-10,000 $^{14}$C a BP (Anderson and Lozhkin, 2015)), North America experienced a rapid increase in temperature (Fritz et al., 2012b; Williams et al., 2001), a decrease in the size of ice sheets

(Stuart, 1991), and major changes in the range and composition of vegetation (Gill et al., 2009; Williams et al., 2001) and animal communities (Gill et al., 2009; MacDonald et al., 2012; Mann et al., 2013). A dramatic global rise in atmospheric $pCO_2$ also occurred at this time (Marino et al., 1992; Martinez-Garcia et al., 2014; Parrenin et al., 2013; Petit et al., 1999). These changes resulted in a significant shift in the structure and composition of the landscape in eastern Beringia (i.e., the North American portion upon which this dissertation is focused). Changes included development of peat lands due to moister and colder summers (Guthrie, 2001), gradual replacement of graminoids and forbs by shrubs and sedges (Hood et al., 2013), restriction of loess deposition and stabilization of sand dunes (Bateman and Murton, 2006), and extirpation of some megafauna, such as steppe bison, horse and woolly mammoth. Humans are also believed to have arrived in the North American Arctic at about this time.

These massive climatic and environmental changes, together with farther climatic fluctuation during the early Holocene, have made the modern region once occupied by eastern Beringia much different from that of the late Pleistocene. The modern vegetation is a mixture of boreal forests, steppe and tundra. Topography, drainage and soils are the most important factors controlling vegetation variety in this ecosystem. Boreal forests, mainly white and black spruce, occupy mesic sites with grassland occupying drier portions (Laxton et al., 1996). Vegetation is sparse and unable to sustain a dense population of mammals (Zazula et al., 2003).

### 1.3.1 Modern Study Areas: Kluane Lake and Whitehorse

While no exact modern analogue has been suggested for late Pleistocene Beringia (Gill et al., 2009; Höfle et al., 2000; Williams et al., 2001), there remain some small portions of west central Yukon (e.g. the eastern shoreline of Kluane Lake) that show some similarities in substrate, climate and vegetation to those of late Pleistocene Beringia (Fraser and Burn, 1997; Laxton et al., 1996). Grasslands located next to the southeast shore of Kluane Lake seasonally receive windblown loess from the Slims River delta. The favorable conditions for loess transportation (katabolic winds, aridity and glaciogenic silt and sand size particles) from the Slims River valley, which resulted from the specific

geographical proximity of the region to the Kaskawulsh Glacier at the center of Kluane Ranges, have produced periods of loess accumulation in the area during late Pleistocene/early Holocene and Neoglacial (Denton and Stuiver, 1966). In most soils from this area, a gap in loess accumulation between these two time periods can be observed as a layer of paleosol named the "Slims soil" (Sanborn and Jull, 2010). A loess base context in this region could somehow resemble that of Beringia during the late Pleistocene that resulted from extensive loess accumulation (Guthrie, 1990).

The modern vegetation of the Kluane region comprises a mixture of grassland and boreal forest (Hoefs et al., 1975). The grasslands are composed mainly of *Artemisia-Festuca* communities, which are predominant in drier parts of the area on southwest-facing aspects (Johansen et al., 1989). The cold and dry continental climate in this region, particularly on south facing slopes, promotes development of grassland communities similar to those that have been suggested for Beringia (Laxton et al., 1996; Zazula et al., 2006a, 2007). Considering the similarities in soil, vegetation and climate, as well as the proximity of this region to the easternmost boundary of Beringia, we consider this region to be as suitable as possible for a modern comparison to Beringia. The south central Whitehorse valley, located farther to the east from Kluane Lake, also has the same continental cold and dry climate and similar grassland vegetation, and thus can be used as another approximate modern equivalent of eastern Beringia. In this dissertation, therefore, both the southeast shore region of Kluane Lake and the south-central Whitehorse valley are used for this purpose. In particular, stable isotope analyses of soil, plants and bone collagen from arctic ground squirrels from these two areas were conducted to establish a modern C and N isotopic baseline for these regions (see Chapters 2 and 3).

### 1.3.2 Klondike Goldfields of West-central Yukon Territory

Northern and southwestern Yukon are located at the easternmost boundary of Beringia. The close proximity of this area to the Laurentide Ice Sheet during the late Pleistocene made it subject to significant climatic, ecological and landscape changes over glacial-interglacial cycles, particularly the most recent (12,500-10,000 $^{14}$C a BP) (Fritz et al.,

2012a). The wealth of ecological records (plant and animal macro- and micro-fossils) recovered from ice- and organic-rich sediments (permafrost) exposed by placer mining in this region provides a great window into a unique record of paleoenvironmental fluctuation during the late Pleistocene (Zazula et al., 2005). The loess sediments were deposited mainly during the last glaciation (after 27k $^{14}$C a BP) (Fraser and Burn, 1997). The recovery of mummified carcasses of different fauna (Kotler and Burn, 2000) and many fossil arctic ground squirrels from these frozen sediments (Zazula et al., 2006a, 2007) also indicate perennially frozen conditions shortly after deposition. Fossil arctic ground squirrel nests recovered at exposures along different gold mines in this region, in particular, provide a useful archive of vegetation for reconstructing the N and C isotopic composition of eastern Beringia during the late Pleistocene. These data, combined with results for the two modern study areas described above, provide an opportunity to test for possible changes in nutrient cycling in this ecosystem and more broadly, significant ecosystem shifts in the region since the terminal Pleistocene.

## 1.4 Main Questions and Organization of the Dissertation

Having a clear image of the late Pleistocene paleoenvironment and nutrient cycling and a good understanding of the foraging ecology, diet and life habits of the now vanished megafauna is needed to understand the potential roles of climatic and environmental changes in their extinction. There is a strong interplay among herbivores, plant nutrient status and ecosystem nutrient cycling (Pastor et al., 2006), and a close coupling between environmental factors and terrestrial N dynamics (Billings et al., 2004; Craine et al., 2009; Frank et al., 2000; Hungate et al., 1997; Williams et al., 2006; Wolf et al., 2010). Therefore, a change in N cycling can be anticipated in Beringia associated with megafauna extinction and major environmental changes at and following the terminal Pleistocene. Evaluation of a possible change in Beringian N cycling is needed to ensure use of an appropriate N isotopic baseline for late Pleistocene Beringia when interpreting and comparing $\delta^{15}$N of fossil bones with modern counterparts (Szpak et al., 2013). Accordingly, a main objective of this dissertation is to determine appropriate C and N food web isotopic baselines for the late Pleistocene in eastern Beringia, and investigate

their possible change over time. Such investigations provide an essential platform for paleodietary studies of megafauna in this ecosystem, including woolly mammoth diet, which is a second focus of this study.

Among late Pleistocene megafauna, the herbivorous woolly mammoth (*Mammuthus primigenius*) – the keystone species in these ecosystems (Church, 2013; Owen-Smith, 1987) – was characterized by anomalously high $^{15}N/^{14}N$ ratios relative to other herbivores (Fig. 1-3). Different reasons have been suggested for this anomaly including: different dietary choices (Schwartz-Narbonne et al., 2015)(Bocherens, 2003; Schwartz-Narbonne et al., 2015), different habitats (Schwartz-Narbonne et al., 2015), physiological differences (Ambrose and DeNiro, 1986; Kuitems et al., 2012) and/or fecal consumption (coprophagy) (Kuitems et al., 2012; van Geel et al., 2011). Given that dietary choices could be one of the important factors in this issue, I hypothesized that the woolly mammoth's plant diet was probably substantially enriched in $^{15}N$ relative to other coeval herbivores' diet. Considering this, the second objective of this project was answering this question that "*why among all herbivores of the late Pleistocene Beringia were woolly mammoths more enriched in $^{15}N$ than other coeval megaherbivores?*" using isotopic analysis of different food sources in this ecosystem.

This thesis addresses this question by testing two main hypotheses. The first hypothesis is that woolly mammoths occupied a different dietary niche to avoid food competition with other herbivores. Considering their specific body shape, including tusks and large legs that provided an ability to disturb soil and snow covers (Putshkov, 2003), the woolly mammoth was probably a specialist herbivore that was selective in its diet. In other words, it occupied a specific dietary niche within the same ecological conditions as other herbivores by feeding on specific plant species or plant parts (such as root crowns) or certain plant materials (decomposed or semi-decomposed) that were more enriched in $^{15}N$. Such behavior could have been particularly important during the late growing season and cold seasons when forage availability was low and plants had lost their above-ground parts. By such times, plants would have transported and stored nutrients from their leaves to their root crowns (Chapman, 1996). Also when senescent plant tissues were covered

under snow or thin soil layers, they likely would have experienced microbially mediated degradation and associated $^{15}N$ enrichment (Connina et al., 2001; Kramer et al., 2003).

The second hypothesis is that the woolly mammoth occupied a different microhabitat from that of most other megaherbivores present in Beringia. In other words, the woolly mammoths foraged within a specific portion of the Mammoth Steppe Ecosystem to preserve their territory and avoid food competition from other megaherbivores. Such behavior has been suggested and observed for other large herbivores in both ancient and modern context, for example for late Pleistocene proboscideans and ground sloths, and modern elk, deer and cattle (McDonald and Pelikan, 2006; Stewart et al., 2002). Therefore, these areas may have become enriched in $^{15}N$ through dung fertilization, which would have constituted a strong feedback affecting soil and plant nutrient contents, N cycling, and hence plant and animal $^{15}N$ contents.

To accomplish the two main objectives of this study, I have taken three main approaches: (i) C and N isotopic analysis of modern soils, plants and arctic ground squirrel bones from the Kluane Lake and Whitehorse areas, which should provide two possible modern comparisons with late Pleistocene eastern Beringia; these data will help to define modern C and N isotopic baselines and describe the possible variation in C and N isotopic compositions of herbaceous food sources; (ii) C and N isotopic analysis of ancient soils, plants and Arctic rodent bones to define late Pleistocene C and N isotopic baselines, and (iii) C and N isotopic analysis of soil and plants in controlled-growth experiments of three common Beringian grasses in Beringian loess soil at the University of Western Ontario's Biotron facility to investigate the contribution of $CO_2$ concentration, dung fertilization and grazing on C and N isotopic variations at the base of food web.

The dissertation is divided into 4 main chapters, excluding the Introduction. Chapter 2 reports C and N isotopic compositions of soil and vegetation from the Kluane Lake and Whitehorse valley study areas, and provide modern C and N isotopic baselines for the regions. Chapter 3 provides analogous analyses for Pleistocene paleosols, plant macrofossils and collagen from fossil bones collected from the perennially frozen loess sediments of Yukon Territory in the Klondike goldfields as well as for modern arctic

ground squirrel (*Spermophilus parryii*) bone collagen for the modern study areas. Thus, comparison between the C and N isotopic compositions at the base of the food web is possible between late Pleistocene and modern times in this region. In Chapter 4, the potential effects of atmospheric $CO_2$ concentration and herbivory on N cycling and vegetation C and N isotopic compositions, both of which changed significantly after the terminal Pleistocene in the study area, are evaluated using growth chamber experiments. Chapter 5 summarizes the collective significance of these results, within the context of the growing body of knowledge concerning the paleoecology of Beringia, and uses these results to explain the high $\delta^{15}N$ of the woolly mammoth.

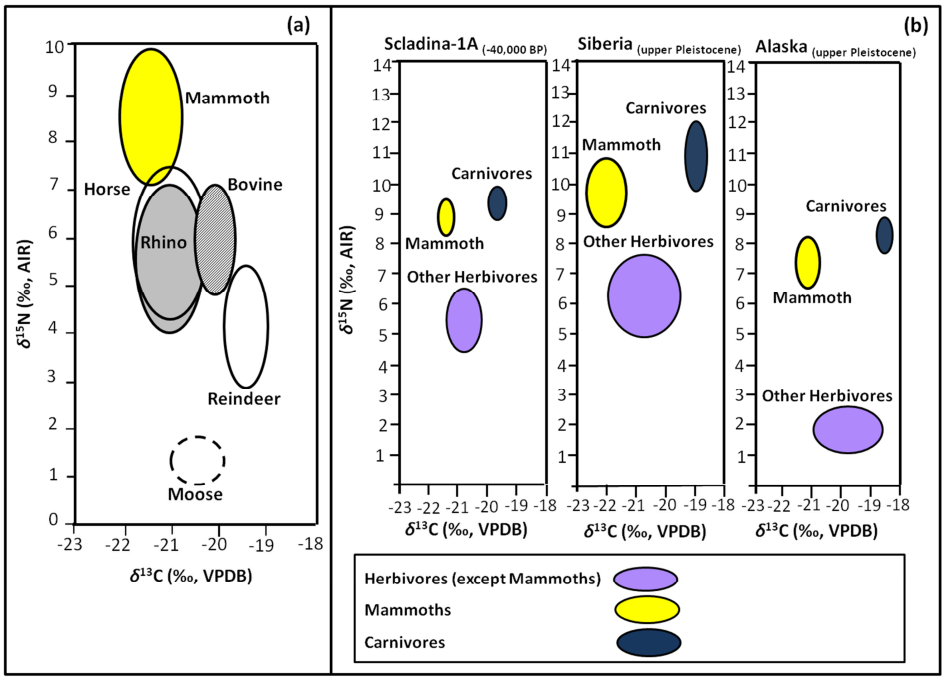


**Figure 1-3: The $\delta^{15}N$ and $\delta^{13}C$ variations of bone collagen from Late Pleistocene mammoth steppe megaherbivores for: (a) entire ecosystem, and (b) Siberia, Alaska and Scladina (Adapted from Bocherens, 2003).**

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# Chapter 2

# 2 Nitrogen and Carbon Isotopic Dynamics of Subarctic Soils and Plants in Southern Yukon Territory: Implication for Paleoecological and Paleodietary Studies

## 2.1 Introduction

Stable isotopes of carbon and nitrogen are particularly valuable for studying food webs and tracking the transfer of energy and materials through trophic levels (Casey and Post, 2011). The range of primary producers in different ecosystems provide varied food sources with distinct carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopic compositions, which comprise the isotopic baseline of ecosystems. Given that $\delta^{13}C$ and $\delta^{15}N$ signatures of any organism reflect both the isotopic composition of its diet and fractionations during the biological processes that build organic tissues, an appropriate isotopic baseline for different ecosystems must be established when studying and comparing animals from different regions and different times (Casey and Post, 2011). This is of particular importance for paleodietary and paleoecological studies of late Pleistocene Arctic and subarctic ecosystems. These ecosystems have experienced frequent high amplitude changes in climate during the late Pleistocene (MIS 5-2, 130 to 10 ka) (Blinnikov et al., 2011), and therefore have a special value in the study of whole ecosystem (structure, composition and function) responses to past climate changes.

Stable isotope analyses have been widely used in the study of late Pleistocene paleoecology of Arctic and subarctic mammals (Bocherens, 2003; Drucker et al., 2011; Guthrie, 1968; Pushkina et al., 2014; Rivals et al., 2010; Stevens and Hedges, 2004; Szpak et al., 2010), their seasonal migration (Julien et al., 2012), their diet (Raghavan et al., 2014) and their extinction (Mann et al., 2013) at different scales. In such work, however, what is most lacking are local and regional isotopic baselines that make interpretation of such data most effective by minimizing errors arising from the assumption that the same isotopic baselines have applied through time and space. The

determination of appropriate modern and past isotopic baselines is necessary for these ecosystems.

Arctic and subarctic ecosystems are not uniform and homogeneous. Converging evidence from multiple proxies such as mammal intestine remains (Kosintsev et al., 2010; Kosintsev et al., 2012; van Geel et al., 2011), plant macrofossils (Wooller et al., 2011; Zazula et al., 2003, 2006), ancient DNA (Willerslev et al., 2014), and pollen (Edwards et al., 2000; Zazula et al., 2006) point to grasses, forbs and sedges as the predominant vegetation types of Arctic and subarctic ecosystems during late Pleistocene. Local and regional ecological mosaics existed because of variation in topography, soil moisture, loess deposition, altitude and animal disturbance (Blinnikov et al., 2011; Zazula et al., 2003). Analyzing local and regional proxies and then combining them at a larger scale should provide a more representative paleoenvironmental reconstruction of these ecosystems (Blinnikov et al., 2011). The modern Arctic and subarctic ecosystems also show this heterogeneity. The presence of bioclimatic subzones, patterned ground created by soil-frost processes, different plant communities, and microhabitats associated with small changes in elevation all produce local and regional differences in these ecosystems (Breen et al., 2014). Such microhabitat diversity should be taken into account when defining modern and past isotopic baselines for ecosystems that are a collage of locally different elements (Guthrie, 1982).

Soils and plants, which can show an extremely wide range of $\delta^{13}C$ and $\delta^{15}N$, are two main components of all terrestrial ecosystems (Amundson, 2003; Craine et al., 2009; Diefendorf et al., 2010). A number of studies have addressed plant $\delta^{13}C$ (Wang and Wooller, 2006; Wooller et al., 2007) and $\delta^{15}N$ (Michelsen et al., 1996; Nadelhoffer et al., 1996; Schulze et al., 1994; Wang and Wooller, 2006; Welker et al., 2003) variations in Arctic and subarctic ecosystems in North America, Eurasia and Iceland. Wooller et al. (2007) conducted the most comprehensive study, analyzing the $\delta^{13}C$ of Herbarium modern plants (around 200 taxa) as well as fossil plants from Alaska and Yukon Territory. Schulze et al. (1994) studied N isotopic and elemental compositions of different plants in northern Alaska with the aim of investigating their difference in

nutrient acquisition. Similar studies have reported $\delta^{15}$N for various plants from other parts of this ecosystem (Michelsen et al., 1996; Nadelhoffer et al., 1996). The analysis of both $\delta^{13}$C and $\delta^{15}$N of plants also has been conducted for *Carex* from 15 Eurasian Coastal Arctic sites (Welker et al., 2003) and for lichens and plants from Iceland (Wang and Wooller, 2006). While these studies add to our isotopic knowledge in these regions, more local and regional isotopic studies are required regarding the extent and heterogeneity of these ecosystems.

In the present study, we measured the $\delta^{13}$C and $\delta^{15}$N of soils and plants from the south of Yukon Territory (eastern shoreline of Kluane Lake and areas around Whitehorse) (Fig. 2-1). The study's purpose is to define a local isotopic baseline for modern food webs and to gain a better understanding of C and N cycling in these regions. The results should provide a good starting point for defining an isotopic baseline for the eastern part of Beringia. Beringia was a largely ice-free land that extended from northwest Canada to northeast Siberia (Höfle et al., 2000) and was located within the Mammoth Steppe Ecosystem, which was the most extensive biome on the Earth during the Last Glacial Maximum (peaking at 18k $^{14}$C a BP; Anderson and Lozhkin, 2015; Zimov et al., 2012). Previous reconstructions of Beringia (Guthrie, 1982; Höfle et al., 2000; Zazula et al., 2003) have shown similarities in climate and main ecosystem contexts (soil and vegetation) to the Kluane Lake and Whitehorse study areas (Laxton et al., 1996).

### 2.1.1 Study Area: Kluane Lake and Whitehorse, Yukon

The two main regions investigated in this study are located in south Yukon Territory: (i) the eastern shoreline of Kluane Lake, and (ii) the Whitehorse area (Fig. 2-1). The first site consists of grasslands located next to the southeast shore of Kluane Lake, which receives windblown loess from the Slims River delta. The second site consists of grasslands in the Whitehorse valley. Table 2-1 lists the mean air temperature and mean total precipitation over the last 29 years at these sites as well as these data for 2012 and 2013, which are the sampling years of this study. A few plant samples were also obtained from three sites in the Faro area (Fig. 2-1) (Table 2-1).

The Kluane Ranges (2000-2800 masl), which are located in the easternmost St. Elias Mountains in the southwest of Yukon Territory, act as a topographic barrier between the northeast Pacific Ocean and the Kluane Plateau (Fig. 2-2). These mountains effectively block penetration of Pacific air masses to the Kluane Plateau, resulting in a semiarid continental climate for Kluane area with cold winters and warm summers (Laxton et al., 1996). Katabolic winds blowing off of the Kaskawulsh Glacier (Hoefs et al., 1975) increase the aridity and make conditions more favorable for loess transport from the Slims River valley within the region. The combination of ice fields at the core of the St. Elias Mountain, from which glaciogenic silt and sand are delivered to the Slims River delta, strong winds off of the Kaskawulsh Glacier, and arid conditions, which amplify evapotranspiration, all facilitate continuous transportation and accumulation of loess on the eastern side of Kluane Lake. Such conditions are similar to those reconstructed by Guthrie (1990) for eastern Beringia loess formation during the late Pleistocene.

Loess accumulation in this area occurred during two time periods: late Pleistocene/early Holocene and Neoglacial (Denton and Stuiver, 1966). The region still experiences frequent dust storms, particularly during summer months (Fig. 2-3) (Muller, 1967). In most soils from this area, the two loess phases are separated by a reddish-brown paleosol (Fig. 2-4), which is named the “Slims soil” and contains a low amount of calcite (Sanborn and Jull, 2010).

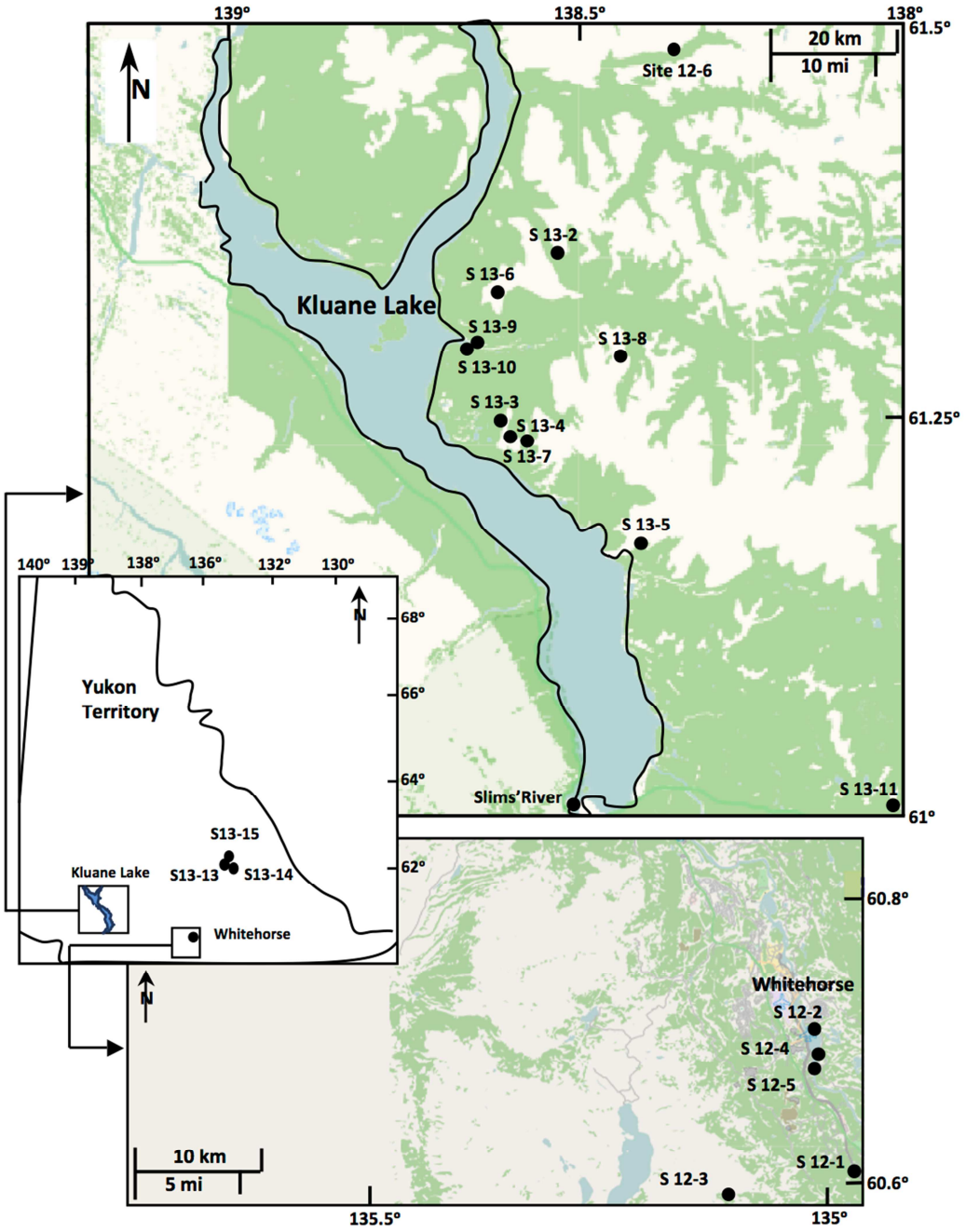


**Figure 2-1: Location of study areas and sampling sites.**

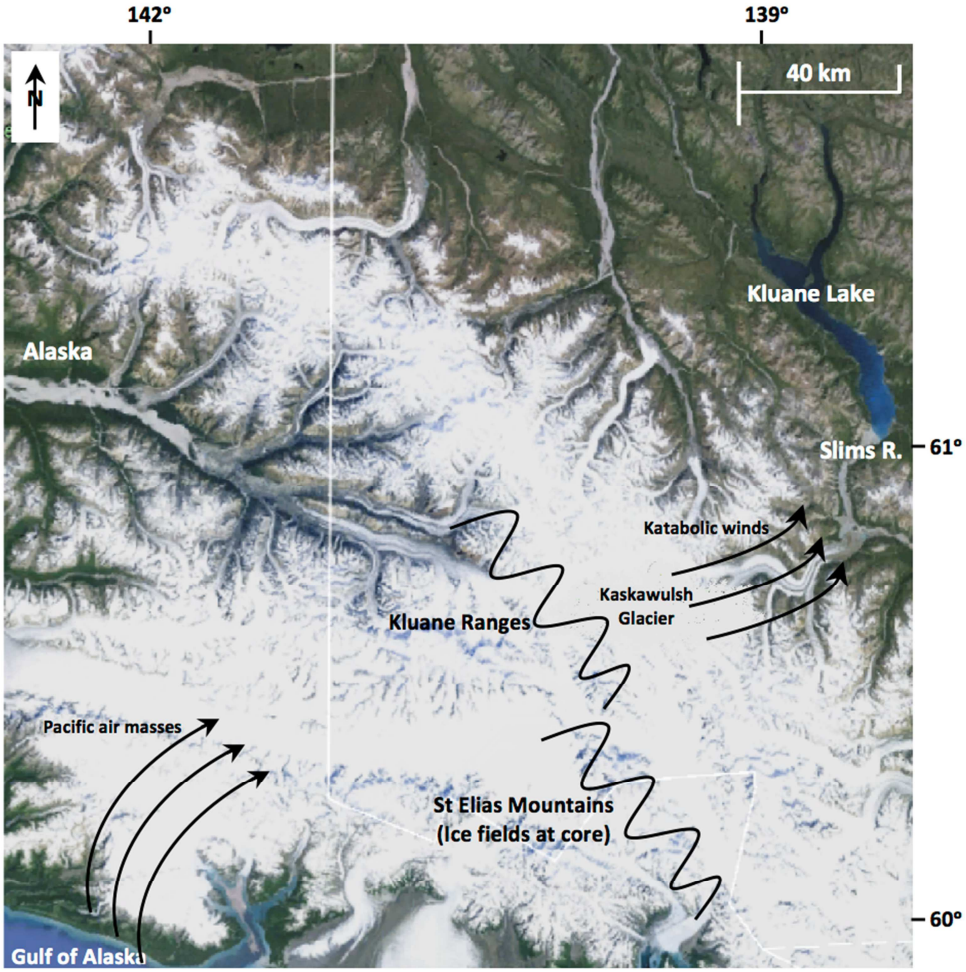


**Figure 2-2: Kluane Ranges, southwest Yukon Territory and Alaska.**

**Table 2-1: Climatic data for Kluane Lake and Whitehorse study areas.**

| | 29 years average (1981-2010) | 2012 (WY)[3] | 2013 (WY) | 2012 (GSM)[4] | 2013 (GSM) |
|---|---|---|---|---|---|
| **MAT (°C) [1]** | | | | | |
| Kluane Lake[5] | -2.1 | -3.0 | -1.1 | 9.4 | 12.0 |
| Whitehorse[6] | -0.1 | -1.0 | 0.5 | 11.1 | 13.3 |
| **MTP (mm) [2]** | | | | | |
| Kluane Lake | 124.3 | - | - | 206.0 | 101.2 |
| Whitehorse | 262.3 | 275.0 | 266.6 | 170.7 | 95.9 |

[1] **MAT**: Mean Air Temperature (Data from Environment Canada, 2015).
[2] **MTP**: Mean Total Precipitation (Data from Environment Canada, 2015).
[3] **WY**: Whole Year.
[4] **GSM**: Growing Season Months (May, June, July and August).
[5] Haines Junction station (60°45'9.7266" N, 137°30'37.5192" W).
[6] Whitehorse station (60°43'59.000" N, 135°05'52.000" W).

**Figure 2-3: Dust storms in Slims River Delta, September 2012 (photograph credit: Fred Longstaffe)**

The vegetation of the Kluane region comprises a mixture of grassland and boreal forest ranging from valley-bottom elevations (781 m) to ~1160 m (Fig. 2-5) (Hoefs et al., 1975). The grasslands are composed mainly of *Artemisia-Festuca* communities, which predominant in drier parts of the area on southwest-facing aspects (Johansen et al., 1989). The forests consist mainly of white spruce (Laxton et al., 1996).

The Whitehorse valley, like most of Yukon Territory, has a dry, subarctic climate (characterized by long and cold winters and short and cool summers). The long-term records (Table 2-1) show a west to east difference in mean total precipitation, with Whitehorse receiving significantly more precipitation than Kluane Lake. The location of the City of Whitehorse in this valley makes its climate milder than other areas of the Yukon (Table 2-1). The vegetation is more or less similar to the Kluane Lake area, consisting mainly of boreal forest and grasslands. Shrub communities are also present near the tree line, under a canopy of trees, especially farther to north on higher plateaus.

Grasslands are limited to dry and south-facing slopes, while forests cover many plateaus and valleys (Cody, 2000).

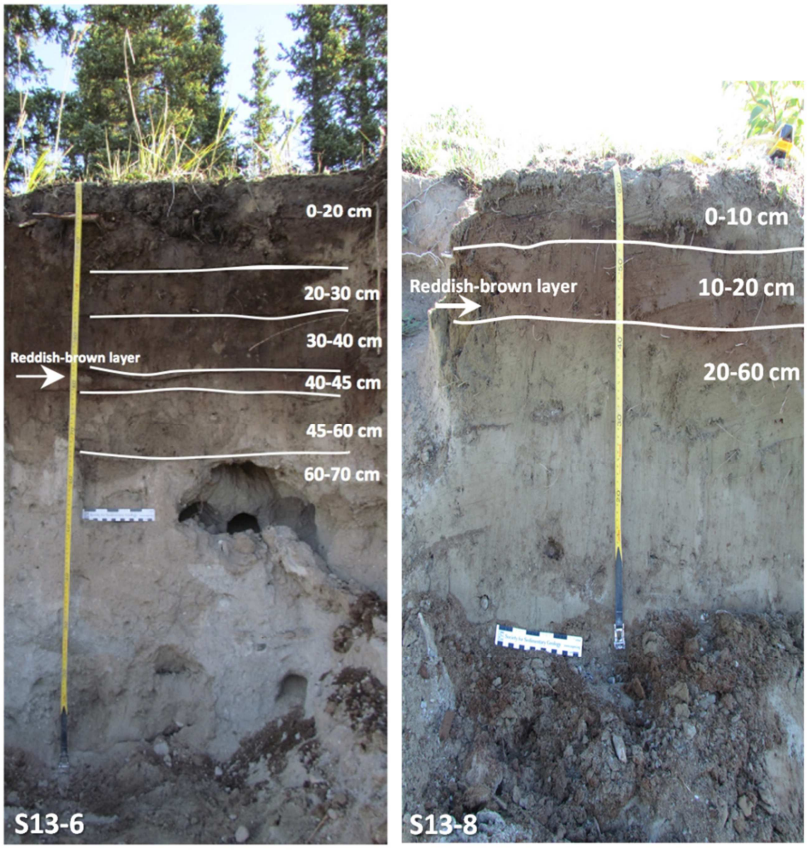


**Figure 2-4: Soil profiles at Kluane Lake (S13-6 and S13-8; photographic credit: Tessa Plint). See Figure 2-1 for site location.**

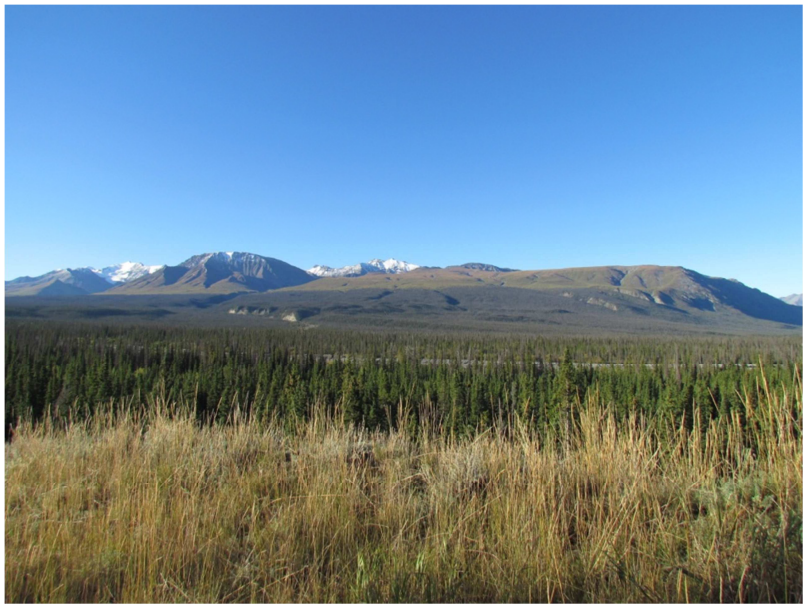

**Figure 2-5: Vegetation cover of the eastern shoreline of Kluane Lake (photographic credit: Tessa Plint).**

## 2.1.2 Carbon Isotopic Composition of Soil and Terrestrial Plants

### 2.1.2.1 Photosynthetic Pathway

The $\delta^{13}C$ of plants is a function of the photosynthesis pathway through which they fix atmospheric $CO_2$. Among vascular plants, $C_3$ plants are characterized by the lowest values (–38 to –22 ‰), $C_4$ plants have higher values (–21 to –9 ‰) and CAM (Crassulacean Acid Metabolism) plants have values intermediate to those of $C_3$ and $C_4$ plants (–30 to –13 ‰) (Tieszen, 1991; Yeh and Wang, 2001). The controls on plant $\delta^{13}C$ have been defined by Farquhar et al. (1989):

Equation 2.1 $$\delta^{13}C_{plant} = \delta^{13}C_{atm} - a - (b - a) \times (C_i/C_a)$$

where $\delta^{13}C_{atm}$ is –8.6 ‰ at the present time (Keeling et al., 2014), *a* is the diffusion fractionation between ambient and intercellular $CO_2$ (+4.4 ‰), *b* is net fractionation by RuBisCO carboxylation (ca. +28 ‰), and $C_i$ and $C_a$ are intercellular and ambient atmospheric $CO_2$ concentrations. Plants that utilize the $C_4$ photosynthesis pathway use PEP carboxylase to fix $CO_2$ from the atmosphere, which discriminates less against $^{13}C$ and results in higher $\delta^{13}C_{plant}$ than in $C_3$ plants (Fig. 2-6). CAM plants (facultative) utilize

both $C_3$ and $C_4$ $CO_2$ fixing pathways, and therefore present a larger range of $\delta^{13}C$ (Cernusak et al., 2013).

These photosynthetic categories have different physiological, biochemical and structural features that cause different responses to environmental factors and therefore result in different geographical distributions. Generally, $C_4$ plants are more dominant in low latitude and tropical regions while $C_3$ plants are more concentrated in high latitudes (Tieszen et al., 1979). All sampling sites in this study are located in high latitudes (above 60°N) and all plants sampled utilize the $C_3$ photosynthesis pathway, as will be demonstrated by their measured $\delta^{13}C$.

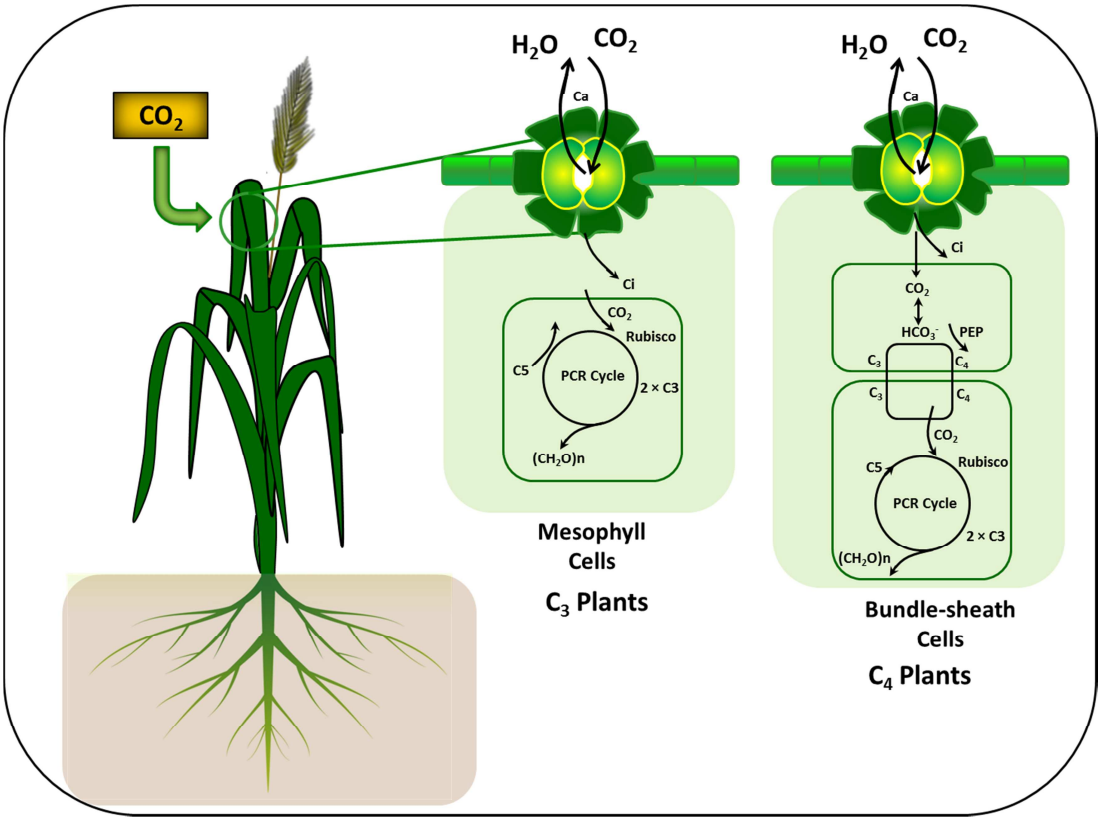


**Figure 2-6: $C_3$ and $C_4$ photosynthetic pathways.**

### 2.1.2.2 Environmental Factors and Plant $\delta^{13}C$

Apart from photosynthesis pathway as the principal control on plant $\delta^{13}C$, there are other environmental factors that can affect plant $\delta^{13}C$ signals including: $\delta^{13}C$ of source $CO_2$, genetics, relative humidity, temperature, light intensity and partial pressure of $CO_2$

($pCO_2$) (Tieszen, 1991; Yeh and Wang, 2001). Generally, these factors affect plant $\delta^{13}C$ by changing the $\delta^{13}C$ of source $CO_2$ or by modifying the $C_i/C_a$ ratio through adjusting the balance between photosynthetic demand for $CO_2$ (enzymatic reactions) and $CO_2$ supply (stomatal conductance) in leaves (Tieszen, 1991).

Water availability appears to be the most important factor that affects plants $\delta^{13}C$ (Murphy and Bowman, 2009). It shows a strong negative correlation with $\delta^{13}C$ in $C_3$ plants but not in $C_4$ plants (Ehleringer and Cooper, 1988; Murphy and Bowman, 2009; Swap et al., 2004). A significant negative correlation between mean annual precipitation (MAP) and $C_3$ plants $\delta^{13}C$ has been reported both locally and globally (Kohn, 2010; Ma et al., 2012; Wang et al., 2008; Weiguo et al., 2005). This relationship has been attributed to stomatal closure of plants in response to water stress and aridity, which results in a reduced $C_i/C_a$, higher water use efficiency and less negative $\delta^{13}C$ (Tieszen, 1991).

Re-assimilation of respired $CO_2$ under dense and closed tree canopies leads to more negative $\delta^{13}C$ due to the fixing of relatively $^{13}C$-depleted $CO_2$ released during soil and canopy respiration (Medina et al., 1986; Van Der Merwe, 1982), and to lower light intensity and higher $pCO_2$ for understory plants (Medina et al., 1991; Sonesson et al., 1992). This phenomenon is referred to as the “canopy effect”. In the present study, no samples collected grew under such conditions, and therefore we do not expect changes in $\delta^{13}C$ arising from a canopy effect.

A latitude effect on plant $\delta^{13}C$ has been reported in a few studies. Stuiver and Braziunas (1987) found a strong negative correlation between latitude and cellulose-$\delta^{13}C$ of North America coniferous trees, which they attributed to the combined effects of changing temperature and humidity. In contrast, Korner et al. (1991) related this isotopic response to combined functions of temperature and atmospheric pressure change with latitude.

A positive correlation between altitude and plant $\delta^{13}C$ has also been observed (Hultine and Marshall, 2000; Korner et al., 1991; Szpak et al., 2013). Although an elevational gradient in leaf structural and chemical characteristics has also been reported (Hultine and Marshall, 2000; Vitousek et al., 1990), the mechanism responsible for this carbon

isotopic response of plants to higher altitude remains unclear. It has been suggested that increased elevation favors photosynthetic $CO_2$ demand over supply through the stomata due to increased carboxylation rate/efficiency (Friend et al., 1989; Korner and Dieme, 1987; Korner et al., 1991). This would decrease $C_i/C_a$ and hence discrimination against $^{13}C$ (Tieszen, 1991).

Irradiation has also been shown to affect plant $\delta^{13}C$, with lower irradiation being associated with more negative $\delta^{13}C$ (Ehleringer et al., 1986; Mulkey, 1986; Zimmerman and Ehleringer, 1990). Less photosynthetic demand for $CO_2$ under low irradiation results in an increase in $C_i/C_a$ ratio and therefore increased discrimination against $^{13}C$. This effect compounds the isotopic signal associated with re-assimilation of $^{13}C$-depleted $CO_2$ under dense canopies (Tieszen, 1991).

#### 2.1.2.3 Intra-plant Variation in $\delta^{13}C$

It is common to see $\delta^{13}C$ variation among different plant parts. Many studies of multiple plant tissues in $C_3$ plants have reported more negative $\delta^{13}C$ in photosynthesizing tissues (e.g. leaf) than heterotrophic tissues (e.g. stem, root, inflorescence) (Brugnoli and Farquhar, 2000; Cernusak et al., 2009; Hobbie and Werner, 2004; Scartazza et al., 1998). This pattern seems consistent in $C_3$ plants, but not in $C_4$ plants (Hobbie and Werner, 2004). Multiple and simultaneous processes have been hypothesized to explain this intra-plant isotopic pattern including different macromolecular compositions of tissues, seasonal variation in photosynthetic discrimination against $^{13}C$ coupled to seasonal variation of macromolecules formation, changing photosynthetic discrimination against $^{13}C$ at the time of leaf expansion, and other processes explained in detail by Cernusak et al. (2009).

Variation in $\delta^{13}C$ of specific organic compounds also has been observed in both $C_3$ and $C_4$ plants, with lignin and lipids being more depleted of $^{13}C$ (Hobbie and Werner, 2004; Park and Epstein, 1961; Wilson and Grinsted, 1976) in contrast to cellulose, sugars and starches, which present less negative $\delta^{13}C$ (Gleixner et al., 1993). A general order of bulk > lipids > *n*-alkanes for $\delta^{13}C$ has been reported for both $C_3$ and $C_4$ plants, with greater

depletion of lipids and *n*-alkanes relative to bulk tissues in $C_4$ than $C_3$ plants (Chikaraisi and Naraoka, 2001; Collister et al., 1994).

In addition to variation among different plant organs and different organic compounds, $\delta^{13}C$ variations also have been observed among different parts of a single organ over different stages of development (Damesin and Lelarge, 2003; Damesin et al., 1997; Leavitt and Long, 1982). This might result from a change in the macromolecular compositions of organs over the growing season as they mature (Damesin and Lelarge, 2003), particularly for leaves that experience a transition from sink organs (organs consuming carbohydrates, very young leaves) to source organs (organs producing excess carbohydrates, primarily mature leaves) over time (Hopkins and Hüner, 2009).

#### 2.1.2.4 Carbon Isotopic Composition of Soil Organic Carbon

The $\delta^{13}C$ signal of soil organic C (OC), which comprises large variety of water-soluble and water-insoluble compounds ranging from simple sugars and carbohydrates to complex proteins, lipids and waxes, is a function of source OC and complex mechanisms that are mainly governed by a complex diversity of soil microorganisms.

Plants are one of the major sources of soil organic matter (SOM). Vegetation using different photosynthesis pathways ($C_3$, $C_4$ and CAM) will therefore impart different $\delta^{13}C$ signatures to their underlying soils (Andreeva et al., 2013). Stevenson et al. (2005) suggested that even small changes in the isotopic composition of plant communities are transmitted to SOM. Many studies have investigated past vegetation and climate change in an ecosystem by measuring the $\delta^{13}C$ of soil bulk OM (Boutton, 1996; Pessenda et al., 1998a-c; Vidic and Montañez, 2004), *n*-alkanes (Zhang et al., 2003) and phytoliths (McInerney et al., 2011) from different soil layers. These studies have interpreted the gradual change in soil $\delta^{13}C$ from deep layers to surface layers as record of the ratio of $C_3$ to $C_4$ plants in earlier vegetation from that ecosystem (Aucour et al., 1999; Francisquini et al., 2014; Freitas et al., 2001).

In addition to the ratio of $C_3$ and $C_4$ contributions to SOM, there are other processes that alter the $\delta^{13}C$ of SOM. Among climatic factors, precipitation has been shown to influence

the $\delta^{13}C$ of soils (Hatté et al., 2001). This can be much more pronounced in soils that retain $\delta^{13}C$ close to those of the original plants from which the SOM formed. A significant negative relationship has been observed between soil organic carbon isotopic ($\delta^{13}C_{OC}$) and MAP in $C_3$-dominated ecosystems (Stevenson et al., 2005). This is explained by physiological and C isotopic responses of plants to MAP, which is then transmitted to soils (Tieszen, 1991).

Discrimination against $^{13}C$ during microbial decomposition is another factor affecting $\delta^{13}C$ of SOM. The extent of this discrimination and its effect on $\delta^{13}C$ depends largely on the magnitude of decomposition, which is in turn controlled by climate (Krull et al., 2002). Many studies explained minor increases in soil $\delta^{13}C$ with depth (2-4 ‰) as a result of microbial degradation of SOM (Bol et al., 1999; Chen et al., 2002; Krull et al., 2002; Nadelhoffer and Fry, 1988), while enrichments larger than 4 ‰ are attributed to $C_3/C_4$ vegetation change (Boutton et al., 1998). Krull et al. (2002), however, argue against this idea. They related SOM enrichments in $^{13}C$ as large as 7 ‰ in the top 20 cm of an Ulitosol from Kakamega forest (tropical region) to fractionation during decomposition, pointing to clay mineralogical and chemical changes to support their supposition.

### 2.1.3 Nitrogen Isotopic Composition of Soil and Terrestrial Plants

In the biogeochemical cycle of N in terrestrial ecosystems (Fig. 2-7), N enters soil from different sources and then undergoes a series of biochemical reactions to continue its active cycling within the soil-plant-atmosphere loop. In contrast to C in plants, which has its main origin in atmospheric $CO_2$, N in plants mainly originates from soil. The $\delta^{15}N$ value of plants, however, is not exactly the same as that of total N (TN) in soil (Makarov, 2009). The difference reflects the range of bioavailable and non-bioavailable N compounds in bulk soil N, and also fractionation of $^{15}N$ at the time of N uptake by plants. Within plants, there are also additional biological processes causing $^{15}N$ discrimination and variability in plants (Evans, 2001; Makarov, 2009).

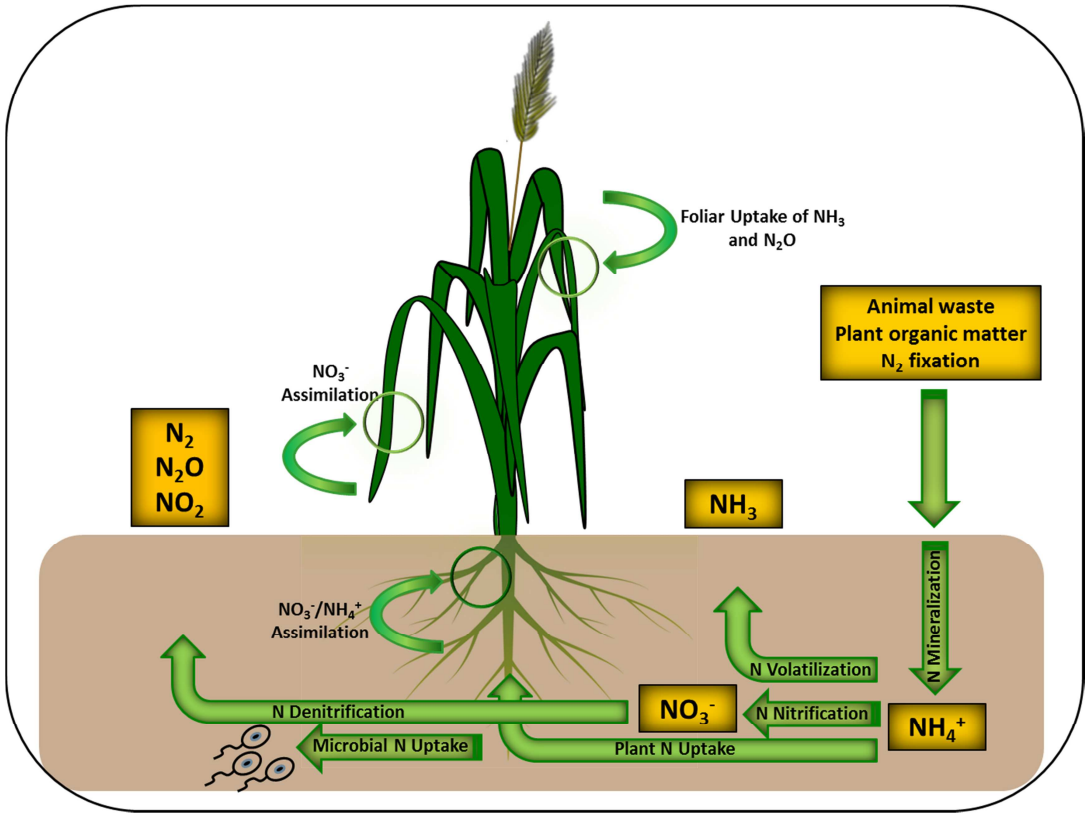


**Figure 2-7: A simple model of N cycling in the soil-plant-atmosphere system.**

### 2.1.3.1 Nitrogen Isotopic Composition of Source N

The $\delta^{15}$N value of soil N is one important factor affecting plant $\delta^{15}$N (Vallano and Sparks, 2012). The most common forms of N in soil taken up by plants are inorganic (nitrate ($NO_3^{2-}$) and ammonium ($NH_4^+$)) and dissolved organic (e.g. simple proteins, amino acids and amino sugars) compounds (Emmerton et al., 2001; Kielland et al., 2006; Näsholm et al., 2009; Schimel and Chapin, 1996; Wei et al., 2013). There are differences in $\delta^{15}$N among these sources because of different biochemical reactions in soil (N mineralization, nitrification, denitrification and volatilization) (Hogberg, 1997; Mariotti et al., 1981). As a result, bulk soil $\delta^{15}$N is not always a good representation of the $\delta^{15}$N of source N to plants because bulk soil might be dominated by non-bioavailable N (Hogberg, 1997).

The quantitative determination of the natural abundance of $^{15}$N across the range of soil sources is very difficult for at least a couple of reasons (Hogberg, 1997; Robinson, 2001).

First, turnover times for both soil inorganic (Davidson et al., 1992; Davidson et al., 1990; Hart et al., 1994) and organic forms (Kielland, 1995) are short, varying from a few days to weeks. Second, the complex dynamics of soil N makes the development of any protocol for accurate extraction and analysis of very small fractions of mobile N forms extremely difficult (Hogberg, 1997; Robinson, 2001). Generally, such studies are performed using $^{15}N$ labeling to identify plant preference for different forms of N (Houlton et al., 2007; Schimidt and Stewart, 1997).

The isotopic fractionation (ε) associated with different N biochemical reactions in soils (Table 2-2) generally result in products more depleted of $^{15}N$ than their substrates (Hogberg, 1997; Makarov, 2009; Robinson, 2001). Accordingly, the following order in $\delta^{15}N$ for different N forms can be predicted:

$$\delta^{15}N_{org} > \delta^{15}N_{NH4}^{+} > \delta^{15}N_{NO3}^{-} \text{ (Makarov, 2009)}$$

While such a pattern has been reported for coniferous soils in Japan (Koba et al., 1998), deviations from this trend are common. The $\delta^{15}N$ signal of the products of soil biological transformations can be higher than their substrate because they can be a substrate for another biological reaction having a higher reaction rate. For example, $\delta^{15}N_{NO3}^{-}$ can be higher than $\delta^{15}N_{NH4}^{+}$ because of a larger degree of denitrification than nitrification (Makarov, 2009).

Another factor adding to the complexity of the isotopic composition of bioavailable N in soils is the $\delta^{15}N$ gradient with soil depth, particularly in natural ecosystems where soils are not tilled (Hogberg, 1997). This can affect plants $\delta^{15}N$ with different root morphologies and rooting depth distribution (Makarov, 2009). A trend of increasing bulk soil $\delta^{15}N$ with soil depth reported in many studies (Hogberg, 1997; Koba et al., 1998; Makarov et al., 2008; Martinelli et al., 1999; Nadelhoffer and Fry, 1988) has been explained by the input of fresh litter depleted of $^{15}N$ in topsoil, and the gradual accumulation of decomposed OM enriched in $^{15}N$ with depth (Makarov et al., 2008).

**Table 2-2: N isotopic fractionation for different biochemical reactions in the N cycle (from Robinson, 2001 and Houlton and Bai, 2009).**

| Process | ε (‰) |
|---|---|
| Microbial $N_2$ fixation ($N_2 \longrightarrow$ Organic N) | 0-6 |
| Nitrification ($NH_4^+ \longrightarrow NO_3^-$) | 15-35 |
| $NH_3$ volatilization ($NH_{4(aq)}^+ \longrightarrow NH_{3(g)}$) | 40-60 |
| Denitrification ($NH_4^+ \longrightarrow N_2/N_2O/NO_2$) | 28-33 |
| Plant $NO_3^-$ uptake and assimilation ($NO_3^- \longrightarrow$ Organic N) | 0-19 |
| Plant $NH_4^+$ uptake and assimilation ($NH_4^+ \longrightarrow$ Organic N) | 9-18 |
| Microbial N assimilation ($NH_4^+ \longrightarrow$ Organic N) | 14-20 |
| Ammonification (mineralization) (Organic N $\longrightarrow NH_4^+$) | 0-5 |
| Nitrate Leaching | 0-1 |

Nonetheless, no regular trend of $\delta^{15}N$ for inorganic N with soil depth has been reported; some studies report an increase in $\delta^{15}N_{NH4}^+$ and $\delta^{15}N_{NO3}^-$ with depth (Koba et al., 1998), while others report a decrease in $\delta^{15}N_{NH4}^+$ with depth over a wide range of alpine and tundra soils (Makarov et al., 2008). Moreover, much evidence supports diversity in the ability of plants to acquire N depending on their rooting depth and phenology (Robinson and Rorison, 1983; Shaver and Billings, 1975), life form (trees, shrubs and herbs) (Schulze et al., 1994) and preference for different forms of N (Houlton et al., 2007; Miller and Bowman, 2002; Nadelhoffer et al., 1996) at different times of the year (Shaver and Kummerow, 1992). In short, a wide range in soil $\delta^{15}N$ among N forms and in the type of N acquired by plants should be considered when interpreting isotopic signals of plants from different ecosystems.

Mycorrhizal fungi association with plants is another factor affecting the $\delta^{15}N$ of source N for plants. Approximately 80 % of terrestrial plants and more than 90 % of vascular plants form biological symbioses with soil mycorrhizal fungi (Wang and Qiu, 2006). This association increases their ability to capture soil nutrients, such as nitrogen (Leigh et al., 2009) and phosphorous (Bolan, 1991). Plants are more reliant on mycorrhizal fungi for N acquisition under conditions of low N availability (Craine et al., 2009). Therefore, plant-

fungi symbiosis is of particular importance in Arctic and subarctic ecosystems where inorganic N is limited (Hobbie and Hobbie, 2008).

Arbuscular mycorrhizal fungi, which mainly colonize the roots of herbaceous plants and tropical trees, play an uncertain role in modifying the $\delta^{15}N$ of plants. It has been suggested that they lack the enzymatic capability to mineralize soil organic N (Hobbie and Hobbie, 2008), and are involved mainly in phosphorous plant nutrition (Craine et al., 2009). In contrast, ectomycorrhizal and ericoidmycorrhizal fungi, which colonizing mainly the roots of trees and shrubs, have the enzymatic capability to dissolve organic N in soils and provide it for plants (Abuzinadah and Read, 1986; Emmerton et al., 2001; Hobbie and Hobbie, 2008). A global survey of plant $\delta^{15}N$ shows that arbuscular mycorrhizal, ectomycorrhizal and ericoidmycorrhizal plants are depleted of $^{15}N$ by 2 ‰, 3.2 ‰ and 5.9 ‰, respectively, relative to nonmycorrhizal plants (Craine et al., 2009; Michelsen et al., 1996, 1998). The cause of such isotopic differences between non-mycorrhizal plants and plants with different types of mycorrhizal associations is not well understood. Some studies explain this pattern by greater uptake of $^{15}N$-depleted organic N from soils by ericoidmycorrhizal plants and greater use of inorganic N by non- and ectomycorhizal plants (Michelsen et al., 1996). Other studies reject this hypothesis and suggest that the $\delta^{15}N$ of soil inorganic N is insufficiently low to explain this pattern. Instead, they attribute lower $\delta^{15}N$ of mycorrhizal plants to discrimination against $^{15}N$ during transfer of N from fungi to host plants (Hobbie and Hobbie, 2006; Schimidt and Stewart, 1997) or to preferential transfer of $^{15}N$-depleted N compounds (e. g. glutamine) produced during enzymatic reactions within fungi to the plant partner (Hobbie and Hobbie, 2008; Hobbie et al., 1999).

There are also a group of bacteria (rhizobia) that make an association with specific groups of plants (legumes) to fix atmospheric $N_2$. This symbiosis affects host plants $\delta^{15}N$ (consistently around 0 ‰) by providing them with N sources having $\delta^{15}N$ close to those of atmospheric $N_2$ ($\delta^{15}N_{AIR}$ = 0 ‰) (Mariotti, 1983). There is negligible fractionation of $^{15}N$ during biological fixation of N by these bacteria (Kohl and Shearer, 1980). None of the plants considered in the present study, however, are from the legume family.

Herbivory activities (grazing, trampling, excretory products and soil disturbance) also can change $\delta^{15}N$ of soil and plants. Herbivory can affect N dynamics in soils and plants by changing N availability (Frank et al., 2000), altering the rate of various soil N processes (Frank et al., 2000; Wolf et al., 2010), and modifying litter quality and plant composition (Augustine and Frank, 2001; Semmartin et al., 2004). These effects have been suggested to be different among different ecosystems, functional groups and species (Augustine and Frank, 2001; Zheng et al., 2012), but no systematic pattern has emerged. Some studies have reported $^{15}N$ enrichment of soils and plants with increased herbivory activities (Aranibar et al., 2008; Coetsee et al., 2010; Frank and Evans, 1997; Li et al., 2010), whereas others report depletion of $^{15}N$ (Frank et al., 2000; Golluscio et al., 2009) or little or no effect (Cook, 2001; Wittmer et al., 2010; Xu et al., 2010).

#### 2.1.3.2 Nitrogen Isotopic Fractionation during N Uptake and Assimilation in Plants

Nitrogen isotope fractionation during plant uptake of $NO_3^-$ and $NH_4^+$ is generally considered to be controlled by two factors: (i) external N concentration (Evans, 2001; Hogberg, 1997; Kolb and Evans, 2003), and (ii) efflux of $^{15}N$-enriched inorganic N and/or $^{15}N$-depleted organic N from roots after N uptake when reduction/assimilation occurs in the roots (Craine et al., 2015; Evans, 2001; Robinson, 2001). Many experiments have reported negligible ε of N during plant uptake under low [$NO_3^-$] and [$NH_4^+$] (~0.5 mol $m^{3-}$), with an increase in ε under higher concentrations (Mariotti et al., 1982; Yoneyama et al., 2001). Because most terrestrial ecosystems are N-limited (Thomas et al., 2013; Lebauer and Treseder, 2008), discrimination against $^{15}N$ during plant uptake is probably negligible under most natural conditions (Evans et al., 1996; Evans, 2001).

Once $NO_3^-$ and $NH_4^+$ are taken up from soils by plants, they undergo various reduction processes to be assimilated. Assimilation of $NH_4^+$ by glutamine synthetase-glutamate synthase (GS-GOGAT) occurs in the roots close to the site of uptake; this arrangement limits toxic accumulation of $NH_4^+$, which interferes with energy metabolism and ATP production (Hopkins and Hüner, 2009). $NO_3^-$ can be assimilated in both roots and leaves through a series of reduction reactions that first reduce $NO_3^-$ to $NO_2^-$ and then $NO_2^-$ to

$NH_4^+$ through the nitrate reductase (NR)-nitrite reductase (NiR) pathways. The $NH_4^+$ then follows the GS-GOGAT pathway to be converted into amino acids and other organic forms of N in plants (Evans, 2001; Hopkins and Hüner, 2009). These enzymatic reactions are the major steps of $^{15}N$ fractionation in plants. Isotopic fractionations of –15 ‰ and –17 ‰ have been reported for NR and GS, respectively (Evans, 2001).

### 2.1.3.3 Intra-plant Variation in $\delta^{15}N$

There are many factors contributing to intra-plant variation of $\delta^{15}N$ including: (i) variation in plant nitrogen sources as different organs form and expand, (ii) different patterns of N assimilation with either $NO_3^-$ or $NH_4^+$ as the primary N source, (iii) reallocation and transportation of N macromolecules between sink and source organs, and (iv) organ-specific efflux of N (Evans, 2001; Szpak et al., 2013).

In contrast to $\delta^{13}C$, which shows more negative values in leaf than stem or root tissues, leaves normally have higher $\delta^{15}N$ than other organs, particularly roots (Evans et al., 1996; Evans, 2001; Yoneyama and Kaneko, 1989). Kolb et al. (2002), however, has reported the opposite pattern, with roots having higher $\delta^{15}N$ than leaves and stems in two species of deciduous trees. When $NO_3^-$ is the sole N source, significant intra-plant variation occurs, with leaves having much higher $\delta^{15}N$ than roots (up to 7 ‰) (Bergersen et al., 1988; Yoneyama and Kaneko, 1989). This likely reflects different patterns of $NO_3^-$ *vs.* $NH_4^+$ assimilation. Whereas $NH_4^+$ is assimilated right after uptake in roots, some $NO_3^-$ is assimilated in roots while the remaining unassimilated $NO_3^-$, which is now more enriched in $^{15}N$, is transported to shoots to be the precursor for N assimilation in leaves (Evans, 2001).

Reallocation of N through different enzymatic reactions and then transport of products to different organs also causes $\delta^{15}N$ intra-plant variations. This occurs because all enzymatic reactions involved should produce molecules with lower $\delta^{15}N$ than the original source (Yoneyama et al., 1998). Loss of $NH_3$ through plants leaves and efflux of organic N from roots also can enrich these organs in $^{15}N$ (Evans, 2001; Shearer and Kohl, 1986).

### 2.1.3.4 Environmental Factors and Plant $\delta^{15}N$

Among different environmental factors, the significant roles of Mean Annual Temperature (MAT) and MAP in controlling soil and plants $\delta^{15}N$ signals have been reported in many studies (Amundson, 2003; Austin and Vitousek, 1998; Craine et al., 2009; Ma et al., 2012). A systematic local and global decrease in soil and plants $\delta^{15}N$ with increasing MAP and decreasing MAT has been observed by Amundson et al. (2003) and confirmed by Craine et al. (2009) for ecosystems with MAT $\geq$ –0.5 $^{o}$C. This relationship may be related to the change in N cycling in soil and plants, the rate of soil N transportation (e.g. denitrification, volatilization) and/or dependence on mycorrhizal fungi (Craine et al., 2009). Changes in the amount of rainfall and subsequently soil water availability can affect the openness of the N cycle (Austin and Vitousek, 1998; Schulze et al., 1991), with a more open N cycle in drier sites most probably reflecting a higher rate of gaseous loss of N (volatilization and denitrification) from soil and plant systems. These reactions leave the soil N pools more enriched in $^{15}N$ due to the large ε associated with them (Table 2-2) (Hogberg, 1997).

One might expect low denitrification rates in drier sites due to the lack of anaerobic conditions required for this reaction. What mainly limits denitrification, however, is $NO_3^-$ availability (Burchill et al., 2014; Groffman et al., 1993), which is not limited in drier sites characterized by more nitrification. Even temporary soil waterlogging after a heavy rainfall can provide favorable conditions for denitrification in drier sites. In addition, lower N availability in wetter sites (Schuur and Matson, 2001), more reliance of plants on mycorrhizal fungi for N acquisition (Craine et al., 2009), and/or a complete conversion of soil $NO_3^-$ to gaseous forms of N through denitrification – which does not leave the system enriched in $^{15}N$ (Houlton et al., 2006) – might contribute to lower soil and plants $\delta^{15}N$ in wetter ecosystems. It has also been suggested that greater N availability in drier sites due to less plant N demand, can stimulate $NH_4^+$ volatilization and therefore higher $\delta^{15}N$ of soil and plant (Austin and Vitousek, 1998). N loss reactions (denitrification and volatilization) should mainly affect actively cycling pools of N, given that identical soil

and plant N isotopic responses have been observed along precipitation gradients (Austin and Vitousek, 1998).

What is clear from these studies is that N cycling in ecosystems, and in turn soil and plant $\delta^{15}$N, are highly responsive and sensitive to climatic factors. Moreover, these changes in $\delta^{15}$N of plants can be tracked along trophic levels from primary producers to consumers (animals and humans) (Schwarcz, 1999; Szpak et al., 2010).

## 2.2 Materials and Methods

### 2.2.1 Sample Collection and Preparation

A total of 79 terrestrial plant samples and 15 soil samples (8 topsoil and 7 subsoil from two soil profiles) were collected during September and August 2012 and 2013 (Fig 2-1). Plant and soil samples were placed in woven poly bags and plastic bags, respectively. At sites S13-8 and S13-10, several topsoil samples were collected due to observed differences in soil texture and the topography of site (downhill with mild slope *vs.* uphill with steep slope). These samples were transported by air to the Laboratory for Stable Isotope Science (LSIS), The University of Western Ontario (London, ON, Canada).

At LSIS, all plant samples were air-dried and then sub-sampled for different plant parts. These plant tissues were then washed with distilled water (DW) and dried at 90$^{o}$C overnight. The dried plant materials were then ground to a very fine powder using a Crescent Wig-L-Bug® and stored in small, sealed glass vials. In preparation for N and C elemental and isotopic measurements, 0.4 mg of each sample was loaded into tin capsules.

All soil samples were air-dried, sieved (< 2 mm), ground gently using a metal mortar and pestle, and then stored in plastic containers prior to physical and chemical characterization. Clay (< 2 $\mu$m), silt (2-47 $\mu$m) and sand (47 $\mu$m-2 mm) fractions (Laxton et al., 1996) were quantified using the hydrometer method (Bouyoucos, 1962) after removal of organic matter (OM) using sodium hypochlorite following Kettler et al. (2001). Soil particles were dispersed for particle size analysis using a probe-type

ultrasonic (probe diameter: 1.27 cm, with 200 watt output) for 1 minute in 0.05 % sodium hexametaphosphate solution. The OM content of soil samples was determined by loss-on-ignition (LOI; 550$^{o}$C for 4 h following Heiri et al., 2001). Soil pH was measured in 0.01 M calcium chloride following Sheldrick (1984). The mineralogy of soil samples was determined using powder X-ray diffraction (pXRD) at LSIS using a Rigaku, high brilliance, rotating-anode X-ray diffractometer equipped with a graphitic monochrometer and CoKα radiation produced at 45 kV and 160 mA. About 1-2 mg samples of soil, which were already oven-dried at 65°C and finely ground using a mortar and pestle, were back-packed into an Al sample holder to achieve random orientation. Samples were scanned from 2° to 82° 2θ at a scanning rate of 10$^{o}$ 2θ/min. The abundance of each mineral was estimated using the unweighted background-subtracted peak height of its most intense diffraction.

Two methods, acid fumigation and acid rinsing, were used to remove carbonates from soil samples prior to elemental and isotopic analyses of OC. In the acid fumigation method following Harris et al. (2001), 30 mg of soil (already ground using a Crescent Wig-L-Bug®), and the range of weights of standards (High and Low Organic Content Sediment and Soil) needed to calibrate a Fisons 1108 Elemental Analyzer, were weighed into silver capsules and placed into a sample tray based in recorded order. Two drops of DW were then added to each capsule and each sample stirred carefully. The sample tray was then placed on a ceramic holder in a glass desiccator above a reservoir containing ~500 ml HCl (12N) and left overnight (at least 18 hours) in a fume hood at room temperature with the desiccator lid half open. The HCl vapor reacted with the wet soil samples in the silver capsules, and dissolved any carbonate present (Brodie et al., 2011; Harris et al., 2001). The sample tray was then removed from the desiccator and placed in an oven at 50-60$^{o}$C until the samples were completely dry. These samples were then wrapped in tin capsules in preparation for measurement of OC content and C isotopic composition.

In the acid rinsing method following Webb et al. (2004) and modified from Brodie et al. (2011), 5 gr of oven-dried samples (65°C overnight) were loaded into 250 ml beakers and

sufficient 1.0 M HCl added to cover the sample. Samples were then stirred on a shaker in a fume hood until the carbonate reaction with acid was complete (30-60 min, depending on the carbonate content of the sample). Sample pH was checked regularly using litmus paper to ensure it remained acidic. Samples were then centrifuged (20 min at 20,000 rpm) and decanted, with the remaining sample being rinsed with DW several times (at least 4) until the sample pH increased to at least 5. The samples were then freeze dried and ground using a pestle and mortar. About 3-30 mg of each sample then was loaded into tin capsules in preparation for measurement of OC content and C isotopic composition.

Untreated soil samples were used to determine total nitrogen (TN) and total carbon (TC) contents, and N isotopic composition because acid-treatment can affect these measures (Harris et al., 2001). About 15-40 mg untreated samples were loaded into tin capsules in preparation for measurement of these parameters.

### 2.2.2 Elemental Analysis

Acid-treated and untreated soils were analyzed for OC, TC and TN contents (dry wt. %) by dry combustion using a Fisons 1108 Elemental Analyzer, which was calibrated using High Organic Content Sediment (accepted value: C = 6.1 wt. %, N = 0.5 wt. %) and Low Organic Content Soil (accepted value: C = 1.5 wt. %, TN = 0.2 wt. %). Sample reproducibility for OC was ± 0.05 wt. % (2 duplicates), ± 0.06 wt. % (6 replicates of High Organic Content Sediment) and ± 0.01 wt. % (6 replicates of Low Organic Content Soil), and for TN was ± 0.01 wt. % (3 duplicates) and ± 0.01 wt. % (6 replicates each of Low Organic Content Soil and High Organic Content Sediment). The elemental data and other information about these reference materials are provided in Appendix A.

The OC and TN contents (dry wt. %) of plant samples were determined using an Elemental Analyzer (EA) (Costech Analytical Technologies, Valencia, CA, USA) coupled to either a Thermo Finnign Delta$^{PLUS}$ XL or a Thermo Finnign Delta V$^{PLUS}$ isotope ratio mass spectrometer (IRMS) (Thermo Scientific, Bremen, Germany). The OC and TN abundances were calibrated using USGS40 (glutamic acid, accepted value: C = 40.78 wt. %, N = 9.50 wt. %) and USGS41 (glutamic acid, accepted value: C = 40.78 wt.

%, N = 9.50 wt. %). Most analytical sessions produced acceptable precision and accuracy for OC and TN contents, as determined using the internal laboratory standard keratin for C and N (accepted values: 48.22 ± 1.07 wt. % (n = 28) for C and 14.85 ± 0.43 wt. % (n = 261) for N) and NIST 1547 (Peach Leaves) for N (certified value: 2.94 wt. %). For some analytical sessions, precision was acceptable (within ± 1 % for C and within ± 0.5 % for N) but accuracy was outside of the acceptable range. For these samples, the results were recalibrated using data from separate analyses performed using a Fisons 1108 EA. Details of this recalibration are summarized in Appendix B. Any samples for which both precision and accuracy were outside of acceptable limits (> ± 1 wt. % for C and > ± 0.5 wt. % for N) were reanalyzed using the Fisons 1108 EA. Samples analyzed using the Fisons 1108 EA were calibrated to the laboratory acetanilide standard ($C_6H_5NH(COCH_3)$: accepted values: C = 71.09 wt. %, N = 10.36 wt. %). The accepted elemental data and other information about these reference materials are provided in Appendix A. All elemental analysis analytical sessions were calibrated to accepted values of standards to two decimal places, with the results being reported to one decimal place considering the SD of replicate measurements.

Following reanalysis of problematic samples, the average C and N contents for keratin standard was 48.13 ± 0.83 wt. % (n = 66) and 14.46 ± 0.49 wt. % (n = 100), respectively, which compare well with their expected values of 48.22 ± 1.07 wt. % and 14.85 ± 0.43 wt. %. Sample reproducibility of duplicates for C was ± 0.33 wt. % (n = 40). The average N content for NIST 1547 was 2.81 ± 0.08 wt. % (n = 50), which compares well with its accepted value of 2.94 wt. %. Sample reproducibility of duplicates for N was ± 0.04 wt. % (n =39).

### 2.2.3 Stable Isotope Analyses

All C and N isotopic results are presented using $\delta$-notation (Coplen, 2011):

Equation 2.2 $$\delta^{13}C \text{ or } \delta^{15}N\ (‰) = [(R_{Sa}/R_{Std}) - 1]$$

where $R_{Sa}$ and $R_{Std}$ denote $^{13}C/^{12}C$ or $^{15}N/^{14}N$ in the sample and standard for $\delta^{13}C$ and $\delta^{15}N$, respectively. The $\delta^{13}C$ and $\delta^{15}N$ of all samples were calibrated to VPDB (carbon)

and AIR (nitrogen) using USGS40 (accepted values: $\delta^{13}C$ = –26.39 ‰ (Coplen et al., 2006), $\delta^{15}N$ = –4.52 ‰ (Qi et al., 2003)) and USGS41 (accepted values: $\delta^{13}C$ = +37.63 ‰ (Coplen et al., 2006), $\delta^{15}N$ = +47.57 ‰ (Qi et al., 2003)). The isotopic and elemental data and other information about these reference materials are given in Appendix A. All analytical sessions were calibrated to accepted values of standards to two decimal places, with the results being reported to one decimal place considering the SD of replicate measurements (see below).

The $\delta^{13}C$ and $\delta^{15}N$ of plant samples and soil OC (after carbonate removal) and TN were measured at LSIS by dry combustion using an EA (Costech Analytical Technologies, Valencia, CA, USA) coupled in continuous flow mode to either a Thermo Finnign Delta$^{PLUS}$ XL or a Thermo Finnign Delta V$^{PLUS}$ IRMS (Thermo Scientific Bremen, Germany). Because of the small amount of N in the plant samples, nitrogen isotopic analysis was performed in a separate analytical session from that of carbon; $CO_2$ generated in these sessions was scrubbed from samples using a Carbo-Sorb trap on the EA. Separate analytical sessions were also used to obtain $\delta^{15}N$ for soil TN, using un-acidified samples, as described earlier. Typical sample sizes for $\delta^{15}N$ measurements were 1-5 mg for plants and 15-40 mg for soils.

Accuracy and precision of the isotopic analyses were monitored using the laboratory keratin and IAEA-CH-6 (sucrose) standards. The average $\delta^{13}C$ obtained for keratin was –24.05 ± 0.09 ‰ (n = 96), which compares well with its accepted value of –24.04 ‰. The average $\delta^{13}C$ value obtained for IAEA-CH-6 was –10.46 ± 0.10 ‰ (n = 33), which compares well with its accepted value of –10.45 ± 0.03 ‰ (Coplen et al., 2006). Sample reproducibility for duplicates was ± 0.10 ‰ for $\delta^{13}C$ (n = 39). The average $\delta^{15}N$ value of keratin was +6.38 ± 0.21 ‰ (n = 108), which compares well with its accepted value of +6.36 ‰. Sample reproducibility for duplicates was ± 0.12 ‰ for $\delta^{15}N$ (n = 27). The elemental results and other information about these reference materials are given in Appendix A. Accuracy and precision results for all standards associated with data presented in this chapter are listed in Appendix C.

### 2.2.4 Statistical Analysis

All plant samples were categorized into two main functional groups: herbs (including annual and perennial grasses, forbs and sedges), and shrub/subshrubs. An independent-sample t-test was used to test for differences in the C and N isotopic and elemental compositions between: (i) different plant functional groups, and (ii) samples from two sampling years (2012 and 2013). Comparisons of C and N isotopic and elemental compositions for (i) different plant parts, and (ii) plants from different sampling sites were performed using one-way ANOVA followed by means comparison using either Tukey's test, if variance was homogeneous, or Dunnett's test, if variance was not homogeneous. Assessment of correlation between (i) plant $\delta^{13}C$ and $\delta^{15}N$, (ii) N content and $\delta^{15}N$ of plant samples, and (iii) soil $\delta^{13}C_{OC}$ and OC in soil profiles were performed using Pearson correlation coefficient. All statistical analyses were performed in SPSS 20.

## 2.3 Results

### 2.3.1 Soils

General information for each sampling site is presented in Table 2-3. Seven topsoils, two soil profiles and one soil sample of the loess source area (Slims River delta) were analyzed for basic physical and chemical properties, and OC and TN isotopic compositions. The results are presented in Tables 2-4, 2-5 and 2-6.

All topsoils, except for S13-9, are dominated by silt (avg. 48.9 ± 16.5 wt. %, all ± errors reported hereafter are one standard deviation or SD). Soil S13-9 contains > 80 wt. % sand. Mean clay concentrations are 18.1 ± 7.8 wt. %. For all samples, there is a strong correlation between OC content from EA measurements and OM content as determined by loss-on-ignition ($R = 0.979$, $p$-value < 0.001). X-ray powder diffraction results show the presence of calcite in most samples (Table 2-5), which is consistent with the average difference between TC of 2.6 ± 1.2 wt. % and OC of 1.8 ± 1.1 wt. % measured for these soils. The near neutral pH (avg. 7.8 ± 0.3) measured for all samples are also consistent with these observations. The mineralogy of most soil samples is similar to that of Slims

River deltaic sediment, which is representative of the sources of windblown deposits in the area (Table 2-5).

The atomic OC/TN ratio of the soils varies from 10.5 to 18.6. The total nitrogen isotope compositions ($\delta^{15}N_{TN}$) of the topsoils range from +2.1 ‰ to +5.5 ‰. The $\delta^{13}C_{OC}$ analysis was conducted on two different groups of pretreated samples for carbonate removal: acid-fumigated and acid-rinsed samples (Table 2-6). All $\delta^{13}C_{OC}$ results derived from both pretreatments are very similar except for samples S13-9 and Slims River for which acid fumigation shows more efficacy in removing carbonates. Accordingly, only results produced using acid fumigation are discussed farther. The range of $\delta^{13}C_{OC}$ (–25.2 to –24.5 ‰) obtained for topsoils is much smaller than measured for $\delta^{15}N_{TN}$, and is characteristic of $C_3$ vegetation. Slims River sediment, by comparison, has $\delta^{15}N_{TN}$ and $\delta^{13}C_{OM}$ of +1.6 ‰ and –20.6 ‰, respectively.

The $\delta^{13}C_{OC}$ of profile S13-6 (Fig. 2-4) increases with depth from –24.5 ‰ for topsoil to –22.5 ‰ in the subsoil (Fig. 2-8a). The change in pH with depth in profile S13-6 strongly correlates with inorganic carbon (IC) content, which was calculated by subtracting OC from TC ($R = 0.960$, $p$-value = 0.002). The $\delta^{15}N_{TN}$ in profile S13-6 also show a positive shift from +4.3 to +6.7 ‰ with increasing depth from topsoil to 60 cm, but then decreases to +4.1 ‰ between 60-70 cm (Fig. 2-8a). A positive, albeit smaller, shift in both $\delta^{13}C_{OC}$ (–25.0 to –24.4 ‰) and $\delta^{15}N_{TN}$ (+4.6 to +5.3 ‰) with increasing depth is also observed for the second soil profile (S13-8) (Fig. 2-4) (Fig. 2-8b).

**Table 2-3: Environmental data for sampling sites and number of soils and plants sampled.**

| Site ID | Site Name | Latitude | Longitude | Altitude (masl) | # of plants | # of Soil Samples |
|---|---|---|---|---|---|---|
| | | | **2012** | | | |
| **S12-1** | Chinook Lane, Whitehorse | 60.5938 | –134.8949 | 728 | 2 | - |
| **S12-2** | Riverdale, Whitehorse | 60.7056 | –135.0343 | 646 | 7 | - |
| **S12-3** | Carcross Road | 60.6213 | –135.0169 | 782 | 5 | - |
| **S12-4** | Schwatka Lake, Yukon River | 60.6724 | –135.0250 | 678 | 3 | - |
| **S12-5** | Miles River Canyon, Yukon River | 60.6614 | –135.0281 | 684 | 7 | - |
| **S12-6** | Destruction Bay | 61.0336 | –138.3666 | 844 | 7 | - |
| | | | **2013** | | | |
| **S13-2** | Cultus Bay, Kluane Lake | 61.3850 | –138.5220 | 783 | 8 | - |
| **S13-3** | Kluane Lake | 61.2581 | –138.6161 | 839 | 11 | - |
| **S13-4** | Kluane Lake | 61.2461 | –138.5650 | 831 | 4 | - |
| **S13-5** | Research Centre, Kluane Lake | 61.1836 | –138.4008 | 781 | 1 | - |
| **S13-6** | Kluane Lake | 61.3530 | –138.6178 | 838 | 8 | soil profile (1) |
| **S13-7** | Kluane Lake | 61.2530 | –138.6019 | 829 | 6 | topsoil (1) |
| **S13-8** | Kluane Lake | 61.3092 | –138.4458 | 821 | 2 | soil profile (1), topsoils (2) |
| **S13-9** | Kluane Lake | 61.3413 | –138.6419 | 813 | - | topsoil (1) |
| **S13-10** | Kluane Lake | 61.3039 | –138.6611 | 805 | 1 | topsoils (2) |
| **S13-11** | Mount Decoli, Kluane Lake | 61.0514 | –137.9867 | 1138 | 4 | - |
| **S13-13** | Robert Campbell Highway | 62.1811 | –133.7603 | 748 | 1 | - |
| **S13-14** | North Canol Road | 61.9972 | –132.3792 | 683 | 1 | - |
| **S13-15** | Faro | 62.3799 | –133.3907 | 1651 | 1 | - |
| **-** | Slims River | 61.0016 | –138.5104 | - | - | topsoil (1) |

**Table 2-4: Soil characteristics.**

| Site ID | Depth | pH | Texture | Sand | Silt | Clay | OM[a] | OC[b] | TC[c] | TN[d] | Atomic OC/TN |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Cm | | | | | | wt. % | | | | |
| **S13-6** | 0-20 | 7.57 | SiL[e] | 29.6 | 57.6 | 12.8 | 7.5 | 2.8 | 3.0 | 0.2 | 17.9 |
| **S13-6** | 20-30 | 7.75 | L[f] | 41.6 | 43.6 | 14.8 | 6.1 | 2.2 | 2.6 | 0.1 | 18.6 |
| **S13-6** | 30-40 | 8.05 | L | 39.6 | 43.6 | 16.8 | 5.7 | 2.1 | 2.9 | 0.2 | 16.2 |
| **S13-6** | 40-45 | 8.18 | L | 39.6 | 39.6 | 20.8 | 6.1 | 2.1 | 3.0 | 0.2 | 15.4 |
| **S13-6** | 45-60 | 8.13 | L | 43.6 | 39.6 | 16.8 | 4.4 | 1.6 | 2.7 | 0.1 | 14.6 |
| **S13-6** | 60-70 | 8.38 | L | 43.6 | 40.0 | 16.4 | 2.9 | 0.5 | 2.2 | 0.1 | 12.9 |
| **S13-7** | 0-10 | 7.54 | SiL | 27.6 | 55.8 | 16.6 | 9.2 | 3.7 | 4.1 | 0.4 | 12.5 |
| **S13-8-1** | 0-10 | 7.73 | SiL | 21.6 | 56.0 | 22.4 | 8.0 | 2.5 | 4.7 | 0.3 | 10.9 |
| **S13-8-1** | 10-20 | 7.99 | SiL | 27.2 | 58.4 | 14.4 | 3.8 | 1.1 | 1.1 | 0.1 | 12.9 |
| **S13-8-1** | 20-60 | 7.72 | SiL | 19.6 | 64.0 | 16.4 | 3.4 | 0.8 | 0.8 | 0.1 | 12.8 |
| **S13-8-2** | 0-10 | 7.52 | SiL | 21.6 | 60.0 | 18.4 | 3.3 | 0.7 | 0.7 | 0.1 | 13.1 |
| **S13-9** | 0-10 | 7.71 | S | 81.6 | 10.0 | 8.4 | 2.1 | 0.5 | 1.8 | 0.1 | 12.6 |
| **S13-10-1** | 0-10 | 7.55 | L | 35.6 | 48.0 | 16.4 | 6.9 | 2.8 | 2.9 | 0.2 | 13.6 |
| **S13-10-2** | 0-10 | 7.25 | L | 37.6 | 46.0 | 16.4 | 8.5 | 3.4 | 4.0 | 0.4 | 10.5 |
| **Slims River** | 0-10 | 7.94 | SiCL[g] | 7.6 | 57.6 | 34.8 | 2.8 | 0.2 | 3.0 | 0.0 | 16.1 |

**[a]: Organic Matter; [b]: Organic Carbon; [c]: Total Carbon; [d]: Total Nitrogen; [e]: SiL: Silty Loam; [f]: L: Loam**
**[g]: SiCL: Silty Clay Loam**

**Table 2-5: Soil mineralogy.**

| Site ID | Quartz | Ca, Na-Feldspar | Calcite | Chlorite/Vermiculite | Amphibole | Mica | Dolomite |
|---|---|---|---|---|---|---|---|
| | wt. % | | | | | | |
| **S13-6 (0-20)** | 69 | 7 | < 5 | 9 | 6 | 6 | < 5 |
| **S13-6 (20-30)** | 70 | 9 | 7 | 7 | 5 | < 5 | < 5 |
| **S13-6 (30-40)** | 60 | 9 | 8 | 5 | < 5 | < 5 | < 5 |
| **S13-6 (40-45)** | 60 | 14 | 9 | 9 | < 5 | < 5 | < 5 |
| **S13-6 (45-60)** | 64 | 17 | 6 | 8 | < 5 | < 5 | < 5 |
| **S13-6 (60-70)** | 68 | 16 | 12 | < 5 | < 5 | < 5 | < 5 |
| **S13-7 (0-10)** | 73 | 19 | < 5 | 6 | < 5 | < 5 | < 5 |
| **S13-8-1 (0-10)** | 41 | 12 | 21 | < 5 | 5 | < 5 | 18 |
| **S13-8-1 (10-20)** | 81 | 15 | < 5 | < 5 | < 5 | < 5 | < 5 |
| **S13-8-1 (20-60)** | 79 | 14 | < 5 | 6 | < 5 | < 5 | < 5 |
| **S13-8-2 (0-10)** | 80 | 14 | < 5 | 6 | < 5 | < 5 | < 5 |
| **S13-9 (0-10)** | 80 | 8 | 6 | < 5 | < 5 | < 5 | < 5 |
| **S13-10-1 (0-10)** | 79 | 39 | < 5 | 8 | < 5 | < 5 | < 5 |
| **S13-10-2 (0-10)** | 80 | 13 | < 5 | 8 | < 5 | < 5 | < 5 |
| **Slims River** | 43 | 8 | 23 | 17 | - | 9 | < 5 |

**Table 2-6: Isotopic composition of soil TN and OC.**

| Sample ID | $\delta^{15}N_{TN}$ (‰, AIR) | $\delta^{13}C_{OC}$ (‰, VPDB) | $\delta^{13}C_{OC}$ (‰, VPDB) |
|---|---|---|---|
| | | Acid-fumigated | Acid-rinsed |
| **S13-6 (0-20)** | +4.3 | **−24.5** | **−24.7** |
| **S13-6 (20-30)** | +6.9 | −24.3 | −24.1 |
| **S13-6 (30-40)** | +7.2 | −23.8 | −23.7 |
| **S13-6 (40-45)** | +7.0 | −23.7 | −23.8 |
| **S13-6 (45-60)** | +6.7 | −23.8 | −23.6 |
| **S13-6 (60-70)** | +4.1 | −22.5 | −23.0 |
| **S13-7 (0-10)** | **+4.0** | −24.9 | −24.8 |
| **S13-8-1 (0-10)** | +4.6 | −25.0 | **−24.4** |
| **S13-8-1 (10-20)** | +4.2 | −24.9 | −25.0 |
| **S13-8-1 (20-60)** | +5.3 | −24.4 | −24.5 |
| **S13-8-2 (0-10)** | +4.8 | **−24.5** | −24.4 |
| **S13-9 (0-10)** | +2.1 | −25.1 | −23.4 |
| **S13-10-1 (0-10)** | +5.5 | −24.5 | −24.5 |
| **S13-10-2 (0-10)** | **+2.5** | −25.2 | −25.1 |
| **Slims River** | +1.6 | −20.6 | −7.5 |

**Values in boldface denote the average result of duplicate analyses.**

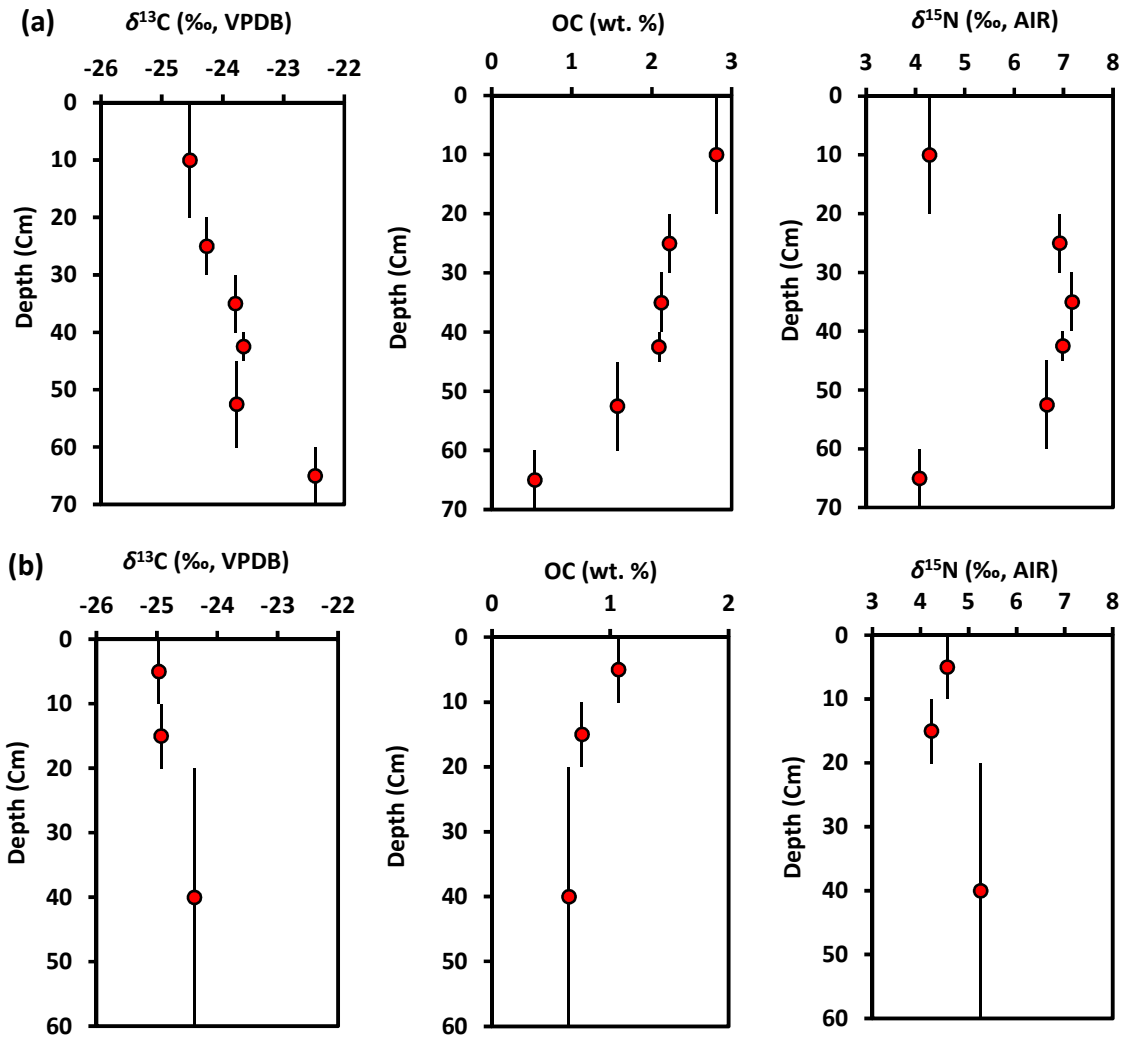


**Figure 2-8: Depth profiles of $\delta^{13}C_{OC}$ and $\delta^{15}N_{TN}$ at (a) sites S13-6 and (b) S13-8 (data points provide average result for soil interval sampled (vertical line)).**

## 2.3.2 Plants

### 2.3.2.1 Isotopic Compositions

A total of 31 (September 2012) and 48 (August 2013) $C_3$ plants representing 15 species (Appendix D) were sampled from 18 sites distributed for the most part just east of Kluane Lake plus some locations around Whitehorse and Faro area, Yukon Territory (Fig. 2-1, Table 2-3). The samples analyzed are from two main functional groups: herbs (including both annual and perennial grasses, forbs and sedges) (n = 66) and shrub/subshrubs (n = 13) (Walker et al., 2005). Subshrubs are smaller than shrubs, but still have a woody base and bushy shape; their soft stems die back during cold seasons.

The $\delta^{13}C$ and $\delta^{15}N$ of leaves (L) and stems (S) were measured for all samples, with additional tissues analyzed when available (70 root crowns (RC), 68 fine roots (FR), 73 inflorescences (I)) (see Appendix D). In this study, the root crown is considered as the top part of the root system in herbs and subshrubs. Subshrubs and perennial grasses regrow each spring from buds produced by the root crown. In the herbs, the major vascular changes required for formation of new stems in spring occur in the root crown (Chapman, 1996).

Foliar $\delta^{13}C$ for all sampled plants ranges from –30.9 to –25.5 ‰, with a mean of –28.0 ± 1.3 ‰ (n = 79). Foliar $\delta^{15}N$ ranges from –8.3 to +7.0 ‰, with a mean of –0.6 ± 2.7 ‰ (n = 77). The foliar $\delta^{15}N$ of two samples (*Artemisia frigida*, Site: S13-2, year: 2013), which are extremely high (+23.4 and +18.7 ‰), have been excluded from this range. Intense volatilization (ε = 40-60 ‰) at the soil surface resulting from urine/dung fertilization by herbivores at this spot could cause such enrichment (Frank et al., 2004).

No significant differences in foliar $\delta^{13}C$ and $\delta^{15}N$ are observed between the two different functional groups (foliar $\delta^{13}C$ of herbs *vs.* shrubs/subshrubs: *p*–value = 0.213; foliar $\delta^{15}N$ of herbs *vs.* shrubs/subshrubs: *p*–value = 0.423), although shrubs and subshrubs have slightly higher mean foliar $\delta^{15}N$ than herbs (+0.1 ‰, n = 11; *vs.* –0.7 ‰, n = 66). Within a given plant, there is a clear pattern of lower foliar $\delta^{13}C$ relative to all other plant parts

(Fig. 2-9), as expressed by the difference in plant part *vs.* leaf $\delta^{13}C$ ($\Delta^{13}C_{\text{plant part-leaf}}$): $\Delta^{13}C_{RC\text{-}L} = 0.5 \pm 0.8$ ‰, $\Delta^{13}C_{FR\text{-}L} = 0.6 \pm 0.8$ ‰, $\Delta^{13}C_{S\text{-}L} = 0.9 \pm 1.1$ ‰, $\Delta^{13}C_{I\text{-}L} = 0.6 \pm 1.4$ ‰. In contrast, no clear pattern of intra-plant variation is observed for $\delta^{15}N$ (Fig. 2-10): $\Delta^{15}N_{RC\text{-}L} = 0.2 \pm 2.5$ ‰, $\Delta^{15}N_{FR\text{-}L} = -0.1 \pm 2.9$ ‰, $\Delta^{15}N_{S\text{-}L} = 0.1 \pm 1.6$ ‰, $\Delta^{15}N_{I\text{-}L} = 0.3 \pm 1.8$ ‰. The range of intra-plant variation in $\delta^{13}C$, however, is smaller than measured for $\delta^{15}N$ (~4 *vs.* 12 ‰).

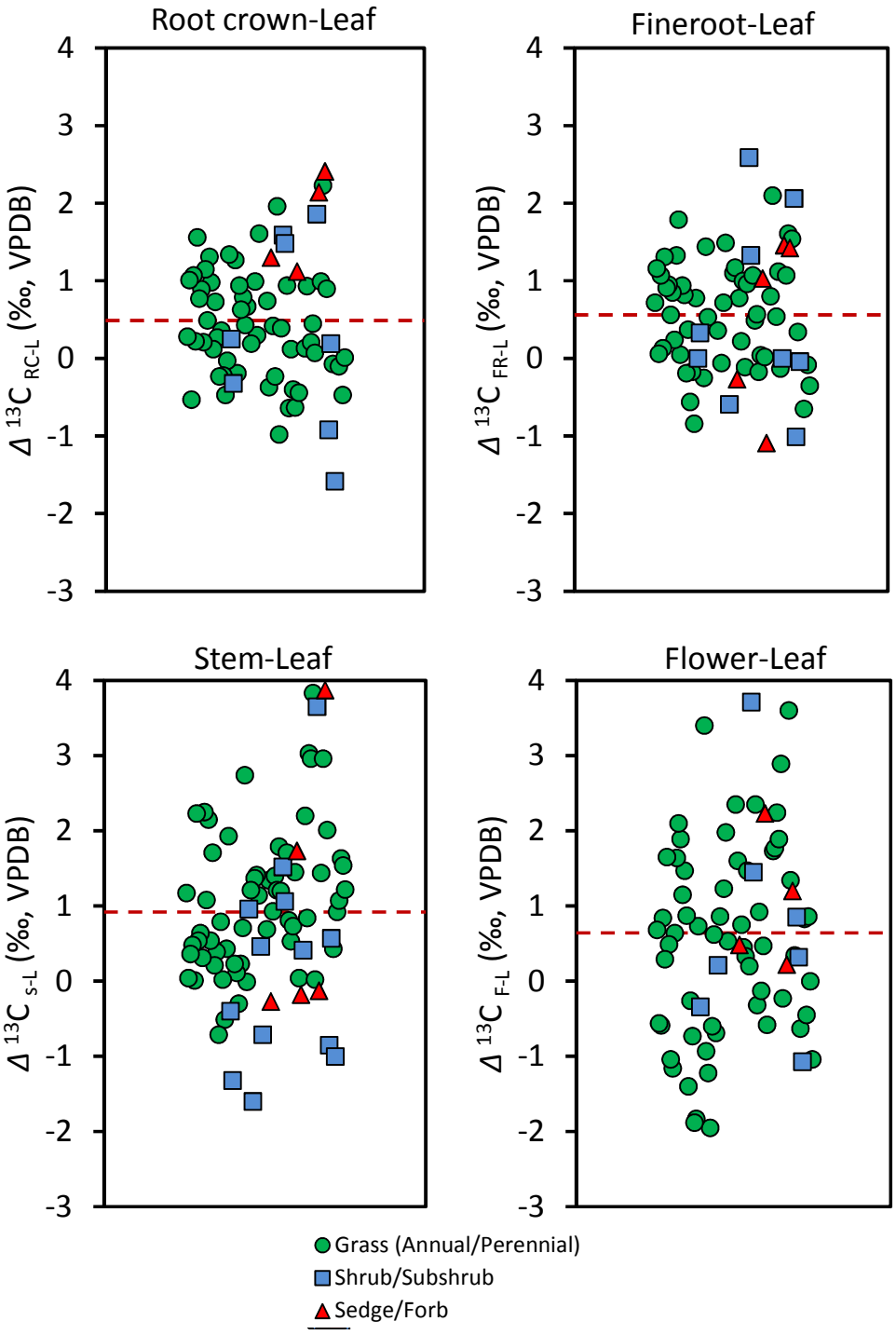


**Figure 2-9: Differences in $\delta^{13}C$ between other plant tissues and leaf ($\Delta^{13}C$). Dashed lines represent means.**

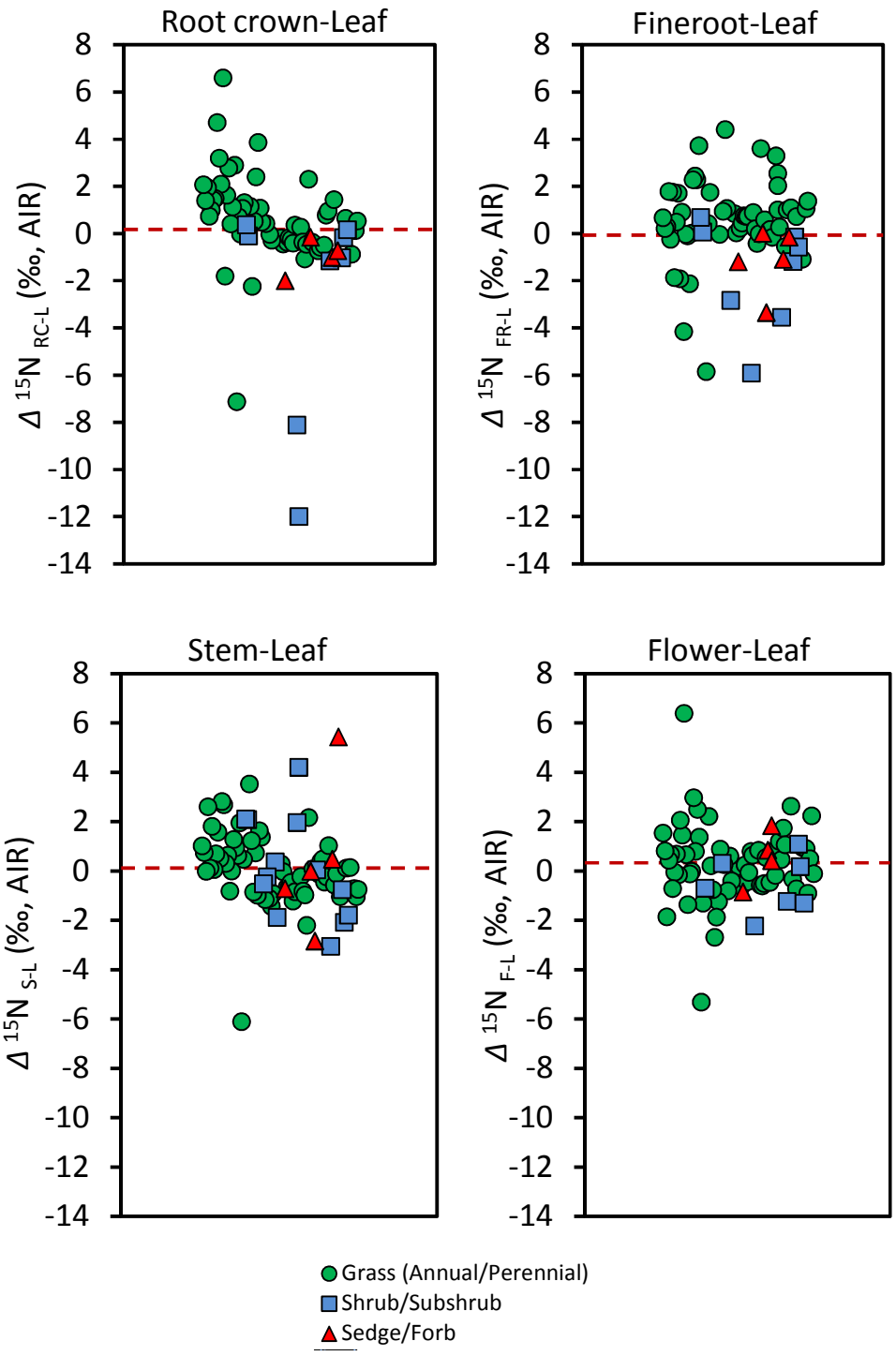


**Figure 2-10: Differences in $\delta^{15}N$ between other plant tissues and leaf ($\Delta^{15}N$). Dashed lines represent means.**

Mean $\delta^{13}C$ and $\delta^{15}N$ for different plant tissues is illustrated for all plants in Fig. 2-11. Below-ground plant parts (root crowns and fine roots) have higher mean $\delta^{15}N$ than above-ground parts (leaves, stems and inflorescences). A one-way ANOVA comparing different plant parts of all plants (Table 2-7) again shows that foliar $\delta^{13}C$ is significantly lower than that of fine roots, stems and inflorescences. In contrast, the difference in $\delta^{15}N$ between below-ground and above-ground plant parts is not statistically significant. There is a positive correlation between foliar $\delta^{15}N$ and $\delta^{13}C$ (R = 0.273, *p*–value = 0.016) for all plant samples from all sites (Fig. 2-12). An independent-samples t-test comparing mean 2012 and 2013 foliar $\delta^{13}C$ and $\delta^{15}N$ show no significant difference between the two sampling years (foliar $\delta^{13}C_{2012}$ *vs.* $\delta^{13}C_{2013}$: *p*–value = 0.604 and foliar $\delta^{15}N_{2012}$ *vs.* $\delta^{15}N_{2013}$: *p*–value = 0.627). A one-way ANOVA comparison of foliar $\delta^{13}C$ and $\delta^{15}N$ among sites with ≥ 5 samples (Figs. 2-13, 2-14) is presented in Table 2-8. There are significant differences in foliar $\delta^{13}C$ between sites S12-5 and S12-2, and sites S12-5 and S12-6 in 2012, with site S12-5 having a lower foliar mean value (–28.7 ‰) than those in sites S12-2 (–26.9 ‰) and S12-6 (–27.3 ‰). In 2013, there are significant differences in foliar $\delta^{15}N$ between sites S13-6 and S13-7 and sites S13-6 and S13-3, with site S13-6 having a higher foliar mean values (+1.4 ‰) than those in sites S13-7 (–2.0 ‰) and S13-3 (–1.3 ‰).

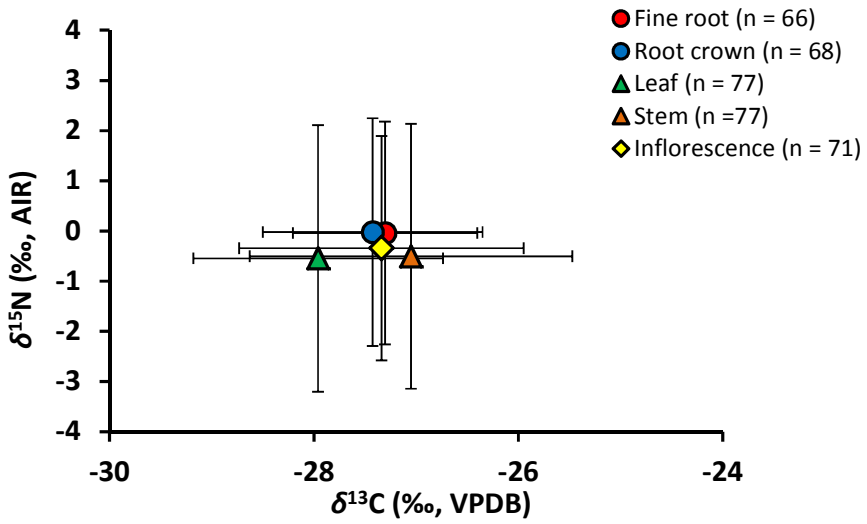


**Figure 2-11: Mean (± SD) $\delta^{13}C$ and $\delta^{15}N$ of all plant parts analyzed.**

**Table 2-7: One-way ANOVA *p*-value results for isotopic differences among all plant tissues.**

| Plant | $\delta^{13}$C (‰, VPDB) | | | | $\delta^{15}$N (‰, AIR) | | | |
|---|---|---|---|---|---|---|---|---|
| tissues | FR | I | L | RC | FR | I | L | RC |
| | | | | | | | | |
| S | 0.922 | 0.930 | **0.001** | 0.616 | 0.785 | 0.994 | 1.000 | 0.754 |
| RC | 0.998 | 1.000 | 0.056 | – | 1.000 | 0.937 | 0.693 | – |
| L | **0.003** | **0.046** | – | – | 0.728 | 0.986 | – | – |
| I | 1.000 | – | – | – | 0.950 | – | – | – |
| FR | – | – | – | – | – | – | – | – |

**FR: Fine root; I: Inflorescence; L: Leaf; RC: Root crown; S: Stem.**
**Values in boldface font are statistically significant ($p \leq 0.05$).**

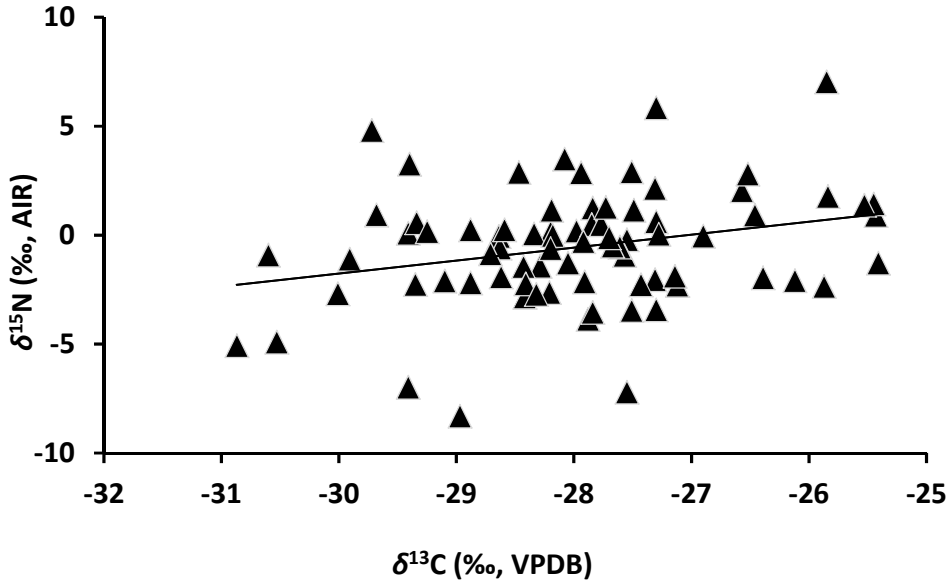


**Figure 2-12: Foliar $\delta^{15}$N *vs.* $\delta^{13}$C for all plant samples.**

**Table 2-8: One-way ANOVA *p*-value results for differences in foliar $\delta^{13}C$ and $\delta^{15}N$ between different sites at which ≥ 5 plants were sampled.**

| | $\delta^{13}C$ (‰, VPDB) | | | $\delta^{15}N$ (‰, AIR) | | |
|---|---|---|---|---|---|---|
| **Sites** | **S12-6** | **S12-5** | **S12-3** | **S12-6** | **S12-5** | **S12-3** |
| | **2012** | | | | | |
| **S12-2** | 0.855 | **0.004** | 0.490 | 0.936 | 0.858 | 0.976 |
| **S12-3** | 0.892 | 0.188 | – | 0.999 | 0.989 | – |
| **S12-5** | **0.027** | – | – | 0.997 | – | – |
| **S12-6** | – | – | – | – | – | – |
| | $\delta^{13}C$ (‰, VPDB) | | | $\delta^{15}N$ (‰, AIR) | | |
| | **S13-7** | **S13-6** | **S13-3** | **S13-7** | **S13-6** | **S13-3** |
| | **2013** | | | | | |
| **S13-2** | 0.889 | 0.269 | | 0.426 | 0.919 | 0.517 |
| **S13-3** | 0.998 | 0.532 | – | 0.715 | **0.037** | – |
| **S13-6** | 0.750 | – | – | **0.011** | – | – |
| **S13-7** | – | – | – | – | – | – |

**Values in boldface are statistically significant (p ≤ 0.05).**

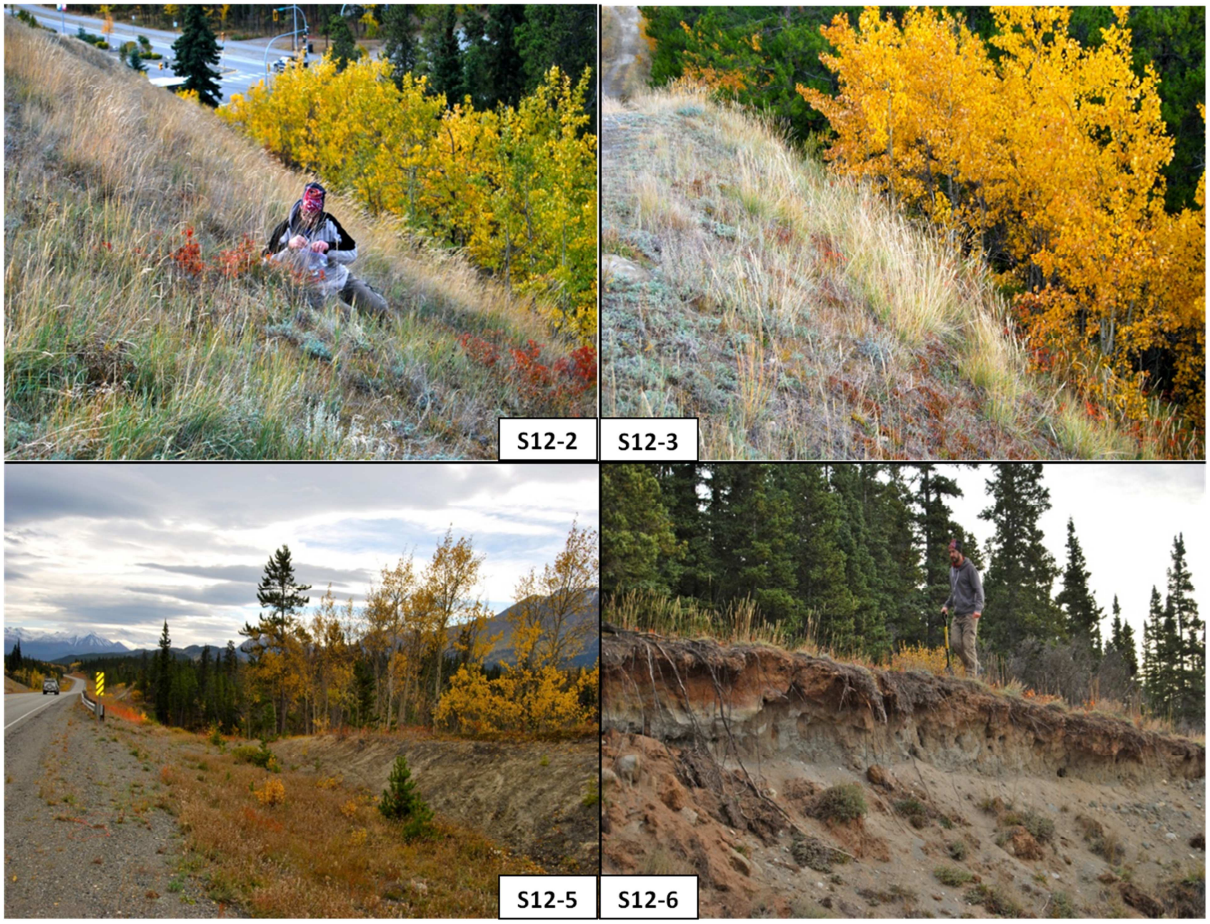


**Figure 2-13: Sites sampled in 2012 (≥ 5 plants) (photographic credits: Fred Longstaffe).**

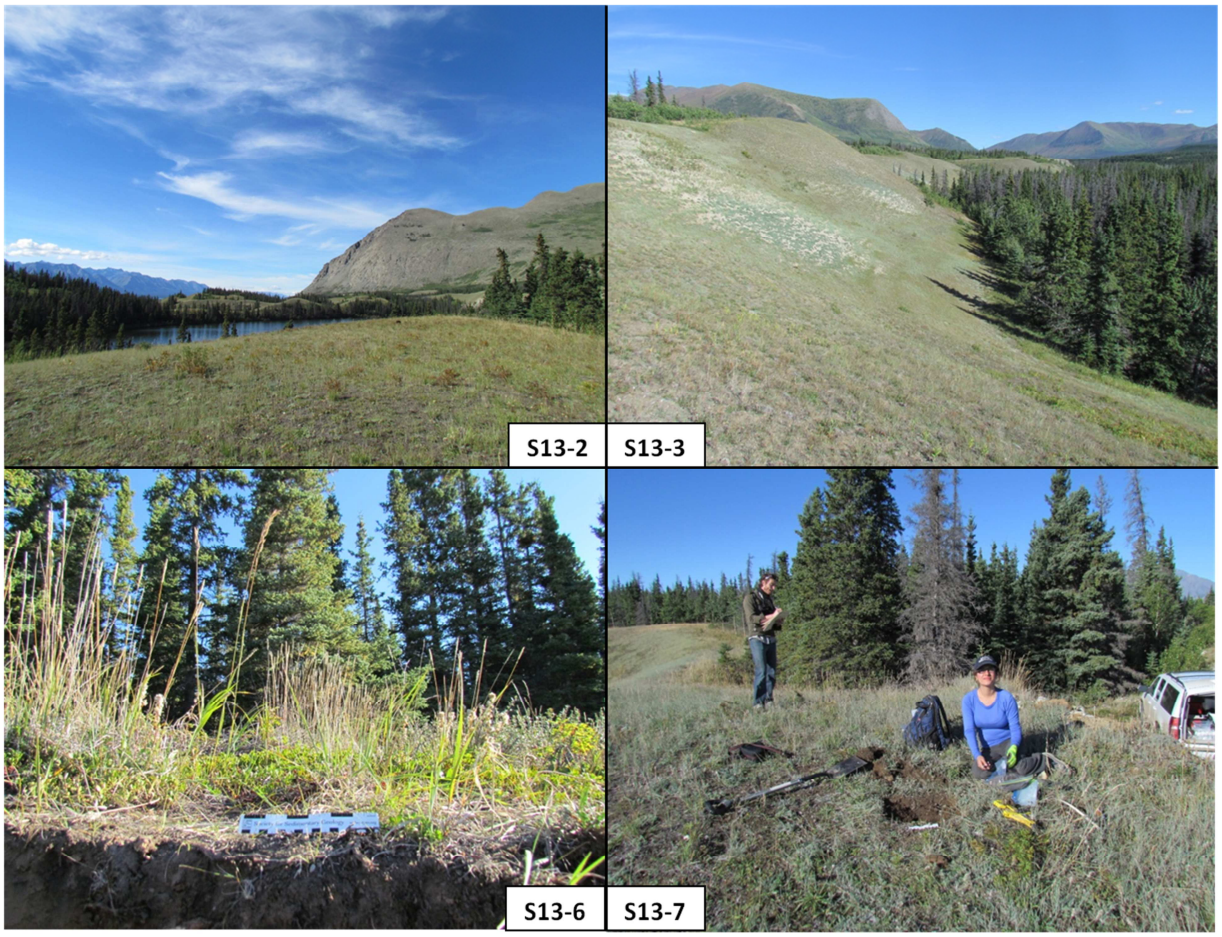


**Figure 2-14: Sites sampled in 2013 (≥ 5 plants) (photographic credits: Tessa Plint).**

### 2.3.2.2 Elemental Compositions

Foliar N contents range from 0.3 to 4.0 wt. %, with an average of 1.1 ± 0.8 wt. % (n = 79). Foliar C contents range from 34.7 to 48.0 wt. %, with an average of 41.3 ± 2.4 wt. % (n = 79) (Appendix E). Foliar atomic C/N ranges from 13.4 to 129.5, and average 61.8 ± 35.5 (Appendix F). In both 2012 and 2013, root crowns have the highest N content (avg. 1.3 ± 0.3 wt. %, n = 70), and stems the lowest (avg. 0.5 ± 0.5 wt. %, n = 79) (Fig. 2-15). Table 2-9 summarizes a one-way ANOVA comparison of C and N contents between different plant parts.

On average, shrubs and subshrubs have higher foliar N and C contents than herbs (Fig. 2-16). There is a significant difference in foliar C and N contents between herbs and shrubs (Table 2-10). A significant positive correlation is observed between foliar (R = 0.389, *p*–value = 0.000) $\delta^{15}$N and N (wt. %) contents among all plants analyzed (Fig. 2-17).

**Table 2-9: One-way ANOVA *p*-value results for differences in C and N contents between different plant tissues.**

| Plant | C (wt. %) | | | | N (wt. %) | | | |
|---|---|---|---|---|---|---|---|---|
| tissues | FR | I | L | RC | FR | I | L | RC |
| S | **0.000** | **0.000** | **0.000** | **0.000** | **0.000** | **0.002** | **0.000** | **0.000** |
| RC | 1.000 | 0.976 | 0.227 | – | **0.000** | **0.000** | 0.209 | – |
| L | 0.103 | **0.011** | – | – | 0.263 | **0.004** | – | – |
| I | 1.000 | – | – | – | **0.043** | – | – | – |
| FR | – | – | – | – | – | – | – | – |

**FR: Fine root; I: Inflorescence; L: Leaf; RC: Root crown; S: Stem.**
**Values in boldface font are statistically significant (p ≤ 0.05).**

**Table 2-10: Independent-samples t-test results for differences in foliar C and N contents between plant functional groups.**

| Functional groups | Shrub and Subshrub | |
|---|---|---|
| | Foliar C (wt. %) | Foliar N (wt. %) |
| Herbs (Grasses, Sedges and Forbs) | **0.000** | **0.000** |

**Values in boldface are statistically significant (p ≤ 0.05).**

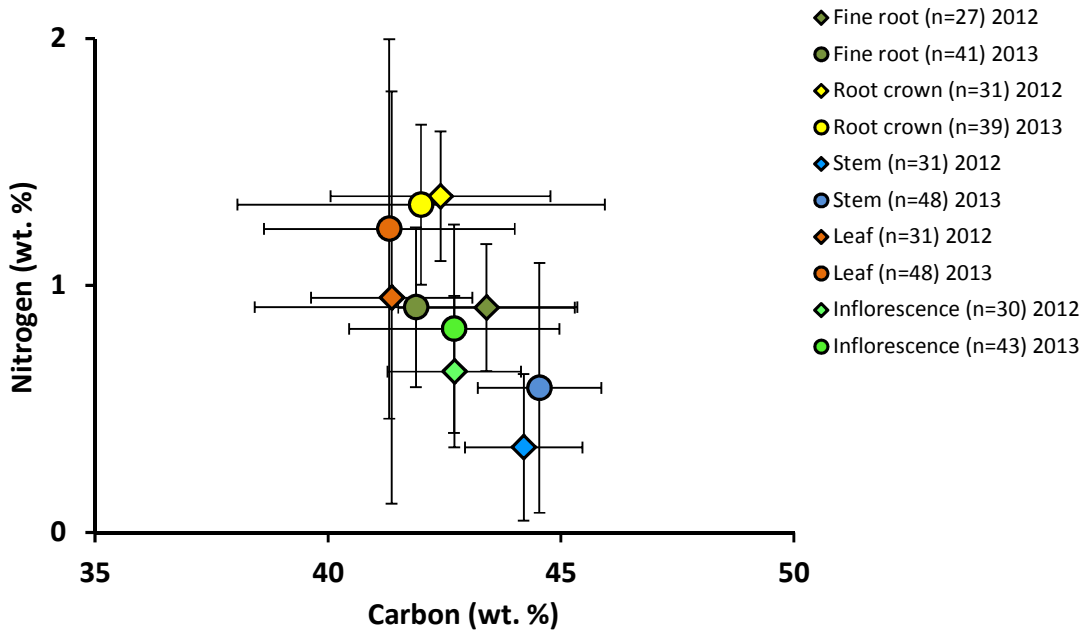


**Figure 2-15: Average N and C contents of plant parts according to sampling year.**

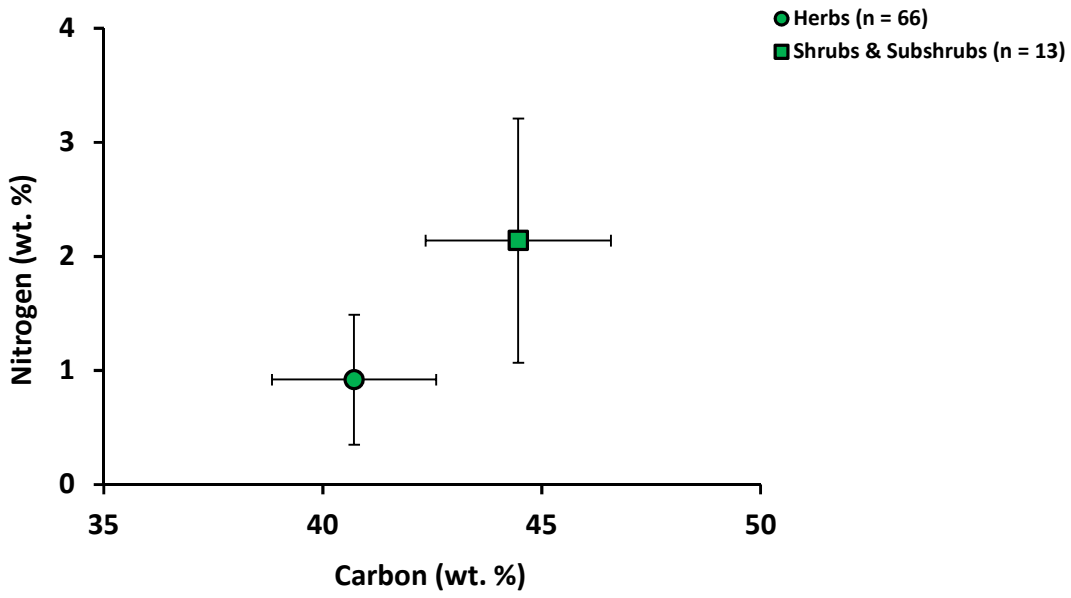


**Figure 2-16: Average foliar carbon and nitrogen contents of different plant functional groups.**

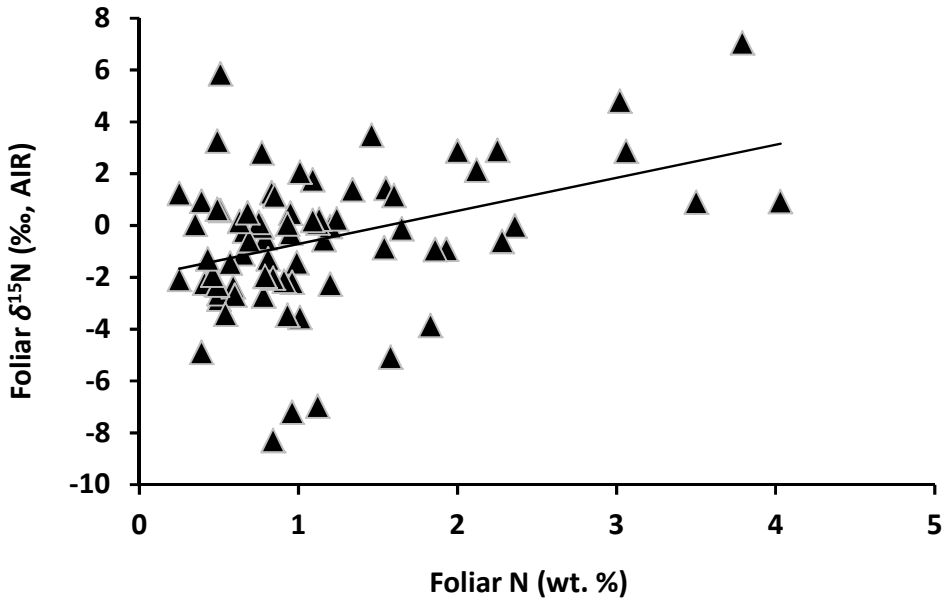


**Figure 2-17: Foliar $\delta^{15}$N *vs.* N (wt. %).**

## 2.4 Discussion

### 2.4.1 Soils of Kluane Lake Area

All topsoil samples studied along the eastern shoreline of Kluane Lake are rich in silty eolian sediment that likely originated from the Slims River delta (Laxton et al., 1996; Sanborn and Jull, 2010) except for S13-9, which contains a larger abundance of sand. The higher sand content at this site likely reflects a larger contribution of underlying sandy glaciofluvial deposits due to bioturbation or post-fire redistribution (Sanborn and Jull, 2010). The soil mineralogy in general shows a strong similarity to the Slims River sediment, which farther suggests a key role for eolian processes in the formation of these soils.

The non-acidic (soil pH > 5.5) nature of the studied soils is characteristic of Arctic tundra settings when the vegetation is dominated mainly by graminoids and forbs (Nordin et al., 2004). The average $\delta^{13}C_{OC}$ (–24.8 ± 0.3 ‰) of all topsoils is typical of SOM generated by $C_3$ vegetation (Desjardins et al., 1996; Pessenda et al., 1998b). The relatively higher $\delta^{13}C_{OC}$ (–20.6 ‰) of Slims River deltaic sediment may point to some contribution of sources with less negative $\delta^{13}C$ such as algae or macrophytes. This site (Figs. 2-18a and

2-18b) is subject to flooding, and probably supports algae and macrophytes at that time. There are both seasonal and diurnal fluctuations in the water flow of the Slims River, which is one of the two main outlets of the Kaskawulsh glacier (Bryan, 1972; Harris, 1990). An ongoing study of aquatic plants in Yukon Territory has found a wide range for foliar $\delta^{13}C_{OC}$ (–41 to –15 ‰), particularly for submerged macrophytes (T. Plint, pers. comm.). Moreover, less negative $\delta^{13}C_{OC}$ (–16 to –13 ‰) for aquatic plants and algae has been reported in continental shelf sediments in Arctic (Naidu et al., 2000). That the higher $\delta^{13}C_{OC}$ reflect contributions of CAM or $C_4$ plants at this site is unlikely, given their insignificant contributions to modern, northern high-latitude vegetation (Naidu et al., 2000). Incomplete removal of carbonate, which would result in less negative $\delta^{13}C_{OC}$ values for the Slims River sample, can be ruled out based on the double test of carbonate removal using both acid fumigation and acid rinsing methods (Table 2-6).

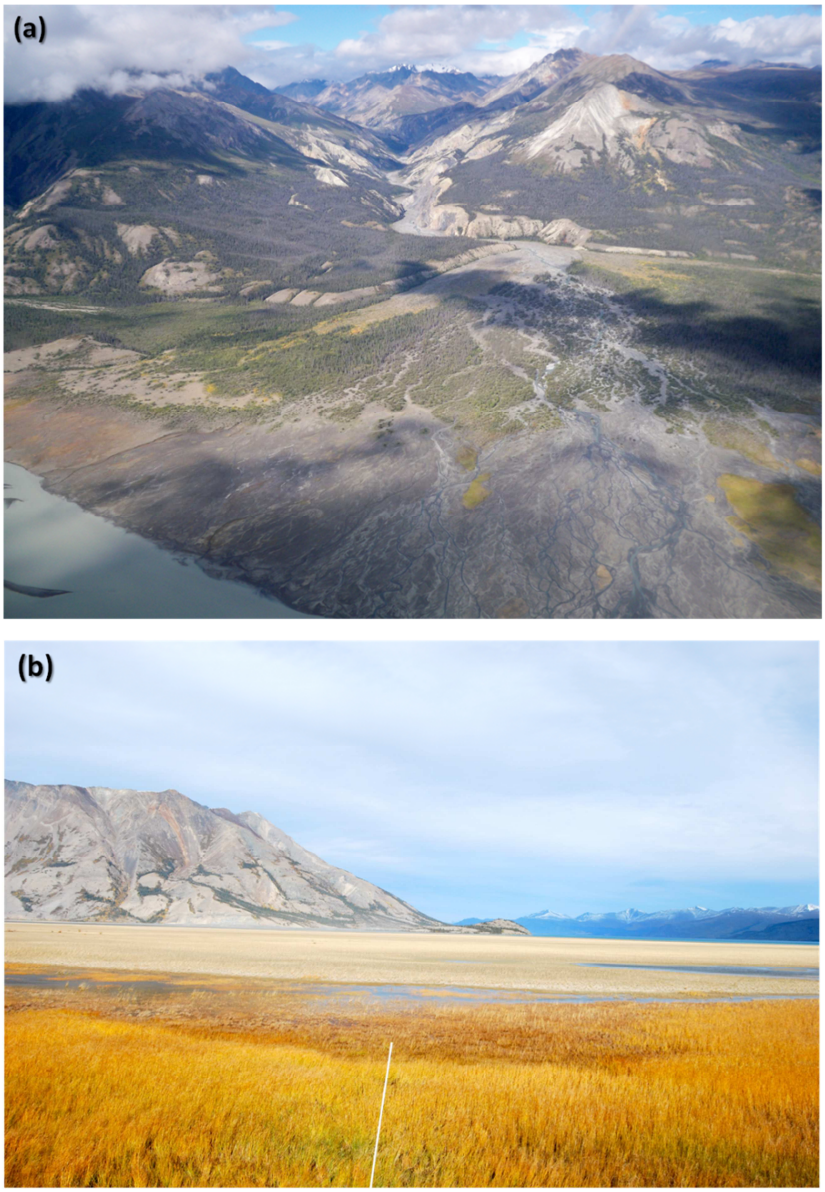


**Figure 2-18: (a) Areal view of the Slims River delta; (b) Slims River vegetation in August (photographic credits: Fred Longstaffe and Tessa Plint)**

In general, all topsoil samples have higher average $\delta^{15}N_{TN}$ and $\delta^{13}C_{OC}$ than plants from the same sites (Fig. 2-19), which is typical of many other terrestrial ecosystems (Nadelhoffer and Fry, 1994; Nadelhoffer et al., 1996; Shearer and Kohl, 1986; Xu et al., 2010). This can be explained by the general enrichment in $^{13}C$ and $^{15}N$ of plant tissues in soil during organic degradation (Chen et al., 2002; Krull et al., 2002; Makarov, 2009).

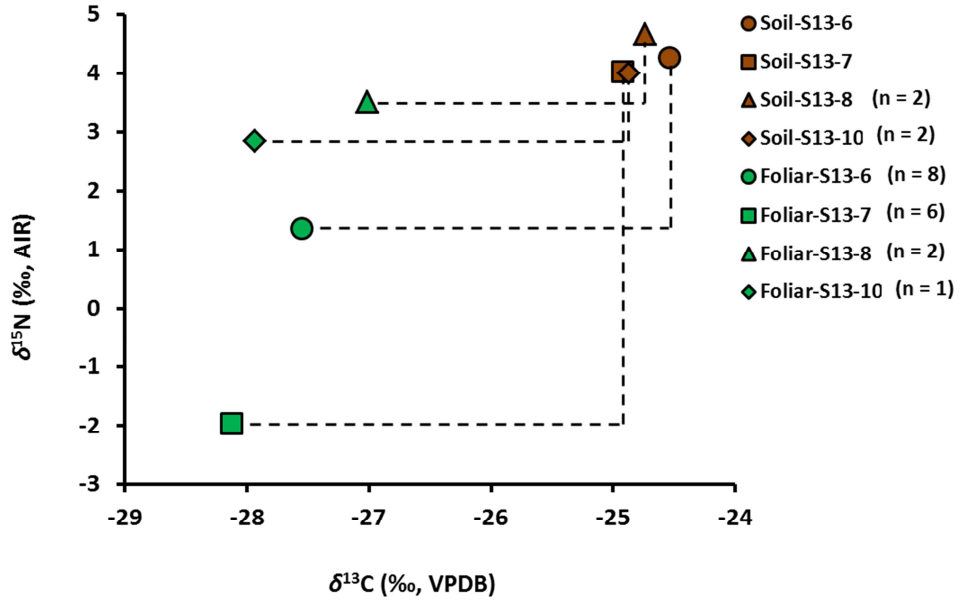


**Figure 2-19: Comparison of topsoil and foliar C and N isotopic compositions.**

The increase in $\delta^{13}C_{OC}$ with depth in the two soil profiles studied is also in agreement with earlier results for subarctic ecosystems (Andreeva et al., 2013; Menyailo and Hungate, 2006). This reflects the input of fresh litter with lower $\delta^{13}C$ at the soil surface and accumulation of decomposed and hence more $^{13}C$-rich OM with depth (see section 2.1.2.4) (Andreeva et al., 2013; Bol et al., 1999; Nadelhoffer and Fry, 1988). This interpretation is consistent with the strong negative correlation between $\delta^{13}C_{OC}$ and OC content (R = –0.950, *p*-value = 0.004) and the decreasing pattern of OC content with depth (Fig. 2-8). There is no evidence for a past vegetation shift in either profile that might explain the change in $\delta^{13}C_{OC}$.

A change in $\delta^{15}N_{TN}$ with depth is also observed in both soil profiles (Fig. 2-8), as has been reported previously for such soils from Siberia (Andreeva et al., 2013; Makarov et al., 2008). The negative shift in $\delta^{15}N_{TN}$ below 60 cm in profile S13-6 may be due to the very small amount of OM (2.9 wt. %) and TN (0.1 wt. %) at this depth, in which case the $\delta^{15}N_{TN}$ signal from inorganic phases may be more important at this depth. In soils, organic N typically has higher $\delta^{15}N$ (+5 to +7 ‰) than inorganic forms ($NO_3^-$ and $NH_4^+$) (–2 to +5 ‰) (Makarov, 2009; Miller and Bowman, 2002) as discussed in section 2.1.3.1.

The $\delta^{15}N_{TN}$ are positive in all topsoil samples, which is typical of alpine and tundra ecosystems (Andreeva et al., 2013; Makarov et al., 2008). Soil $\delta^{15}N_{TN}$ > 0 ‰ point to inputs with compositions higher than produced by fixation of nitrogen from air and/or N-loss processes that leave the soil N pool enriched in $^{15}N$ (Wang et al., 2014). Two samples (S13-9, S13-10-2) sit at the lowest end of N isotopic range (+2.1 to +5.5 ‰). S13-9 has the highest sand and lowest clay and silt contents of the soils examined in this study. The content, structure and function of OM associated with mineral particles generally vary with grain size. Sand-sized particles are typically associated with less humified and lower abundances of OM than silt and clay (Christensen, 2001). Several studies of soils underlying grasslands have reported higher $\delta^{15}N_{TN}$ for clay-sized fractions (~+9 to +12 ‰), which generally have a higher content of stable, humified OM than silt (+5.5 to +9 ‰) and sand (+2 to +7 ‰) particles (Ledgard et al., 1984; Nacro et al., 2004; Tiessen et al., 1984). Similar observations have been reported for soils underlying a pine and oak forest in southern California (Quideau et al., 2003).

Topography may explain the low $\delta^{15}N_{TN}$ (+2.5 ‰) of sample S13-10-2 at site S13-10. Sample S13-10-2 was collected from top of a steep slope (Fig. 2-20), which made it more susceptible to erosional disturbance, while sample S13-10-1 ($\delta^{15}N_{TN}$ = +5.5 ‰) (from the same site) was collected at the bottom of the slope from a flat and more stable location. Amundson (2003) suggested that steeply sloping soils can have $\delta^{15}N$ close to atmospheric inputs because of continuous soil removal and SOM rejuvenation, which maintains the soil's N status far from steady state.

**Figure 2-20: Site S13-10 (photographic credit: Tessa Plint).**

### 2.4.2 Plant C and N Isotopic and Elemental Compositions

The range of $\delta^{13}C$ (–32.5 to –23.5 ‰) measured for all plant parts in this study (Fig. 2-21) is typical of $C_3$ vegetation, which dominates high latitude ecosystems (Sage et al., 1999). Variation in environmental factors (slope aspect, light, water availability and topography) even within small microhabitats may cause this large spread in $\delta^{13}C$. Wooller et al. (2007) reported identical range of foliar $\delta^{13}C$ for sedges and grasses from Alaska and Yukon Territory.

The plants analyzed here show much more variation in $\delta^{15}N$ (> 10 ‰), ranging from negative to highly positive (Fig. 2-21), consistent with results previously reported for Arctic and subarctic regions (Michelsen et al., 1996; Nadelhoffer et al., 1996; Schulze et al., 1994). Variation in $\delta^{15}N$ of vegetation tends to be more pronounced in N-limited ecosystems like the Arctic and subarctic, and points to utilization of different soil N resources by plants depending on their life forms, type of mycorrhizal association and rooting depth and morphology (Nadelhoffer et al., 1996). Coexisting plant species are known to partition N resources with different $\delta^{15}N$ in these ecosystems (McKane et al., 2002; Miller and Bowman, 2002; Nadelhoffer et al., 1996; Schulze et al., 1994). For example, the grass *Calamogrostis canadensis* ($\delta^{15}N$ = +0.9 ‰) in Alaska acquires N from

deeper soil horizons, while the evergreens *Picea glauca* and *Picea mariana* (–7.7 ‰) likely utilize ammonium or organic N from fresh litter (Schulze et al., 1994).

There are likely five main environmental factors that control the isotopic compositions of the herbaceous plants, shrubs and subshrubs sampled in this study: (i) water availability, (ii) N availability, (iii) spatial differences, (iv) plant functional groups, and (v) intra-plant variation. Each factor is discussed next.

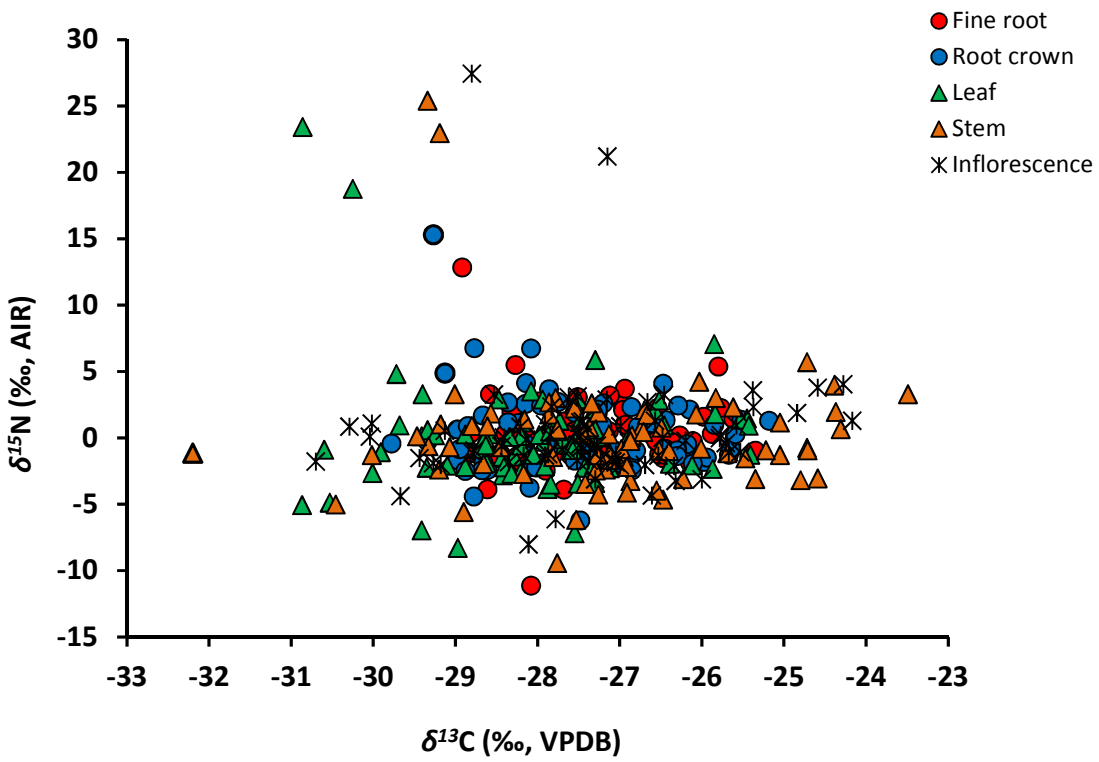


**Figure 2-21: Nitrogen *vs.* carbon isotopic compositions of all plant parts.**

### 2.4.2.1 Water Availability

The weak positive correlation observed between foliar $\delta^{15}N$ and $\delta^{13}C$ of all samples analyzed may point to the importance of water availability as a driving factor in changing and controlling both $\delta^{15}N$ and $\delta^{13}C$ of plants in this ecosystem. Plants capture atmospheric $CO_2$ through leaf stomata and fix it using enzymatic reactions, while N is mainly obtained through roots from soil or symbiotic associations and then assimilated. Given that the sources and pathways determining the $\delta^{13}C$ and $\delta^{15}N$ of plants are

different, the observed correlation likely indicates an environmental factor driving both isotopic signals in the same direction. As discussed in the Introduction, a change in MAP and therefore water availability can change both $\delta^{13}C$ and $\delta^{15}N$ of plants in the same direction (Fig. 2-22) (N: Amundson, 2003; Craine et al., 2009; Martinelli et al., 1999; C: Ehleringer and Cooper, 1988; Murphy and Bowman, 2009; Swap et al., 2004; Weiguo et al., 2005). The pattern observed in the present study therefore supports the idea that water availability is a factor in determining the isotopic composition of plants in this region.

Ma et al. (2012), in particular, have reported an aridity-associated positive correlation between $\delta^{13}C$ and $\delta^{15}N$ in plants from northern China and noted that plant isotopic sensitivity to water availability can vary among different ecosystems and even plant species. Such ecosystem- and species-specific variations in isotopic responses to water availability is likely an important factor in paleodietary and paleoecological reconstructions of Arctic and subarctic regions, and warrants farther investigation.

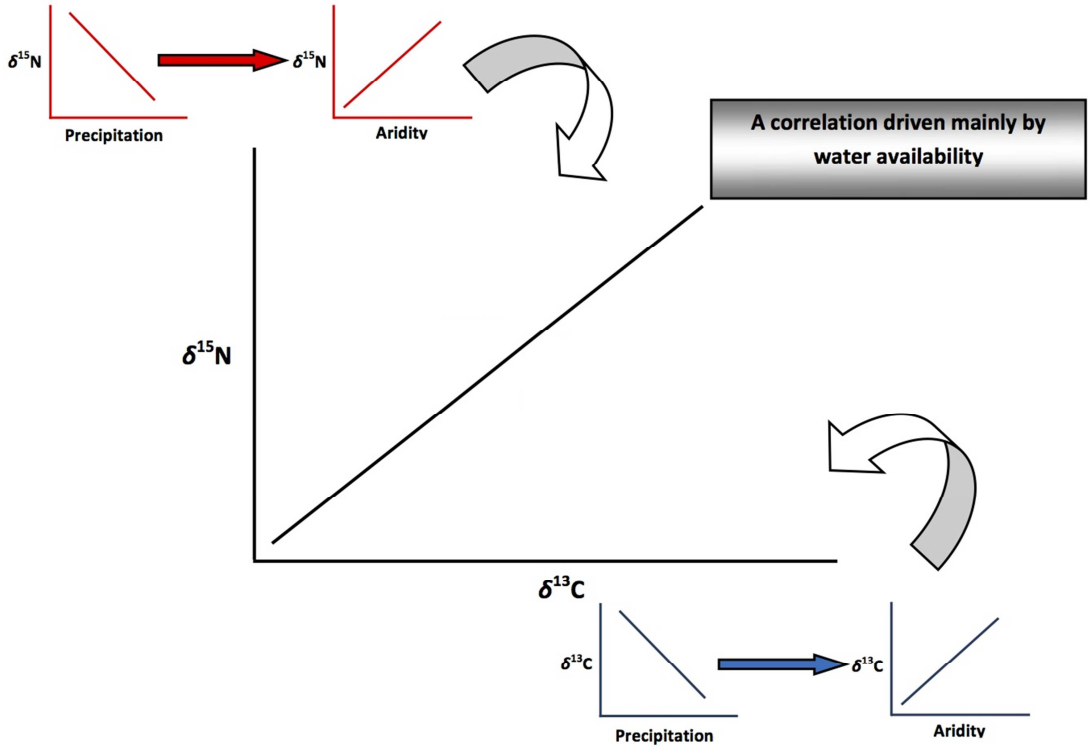


**Figure 2-22: A simplified model to explain a positive correlation between plant $\delta^{15}N$ and $\delta^{13}C$.**

#### 2.4.2.2 N Availability

The positive correlation between foliar $\delta^{15}N$ and N content suggests a key role for N availability and N cycling in determining the N isotopic signal acquired by plants in this ecosystem. Such a correlation has been reported previously on local (McLauchlan et al., 2010; Schulze et al., 1994), regional (Craine et al., 2005; Martinelli et al., 1999) and global (Craine et al., 2009) scales.

Higher plant $\delta^{15}N$ reflects higher N availability and a more open N cycle in terrestrial ecosystems (Hietz et al., 2011; Hogberg, 1997; McLauchlan et al., 2007, 2010, 2013; Pardo et al., 2006). The globally observed positive correlation between soil and foliar $\delta^{15}N$ suggest foliar $\delta^{15}N$ as an index for N availability in soils and therefore ecosystems (Craine et al., 2009). Nonetheless, the best way to describe N availability for plants in different ecosystems remains unclear (Craine et al., 2009). It can be defined in several ways including: (i) an increase in N inputs into the soil from different sources (animal dung, plant materials, microbial N fixation), (ii) increased OM decomposition and N mineralization, (iii) increased $NO_3^-$ production through more nitrification (Pardo et al., 2006), and (iv) less N demand by plants, mainly in drier localities (Austin and Vitousek, 1998). In any of these scenarios, higher inorganic N availability in soils means that extra N is available to fuel N loss processes (e.g. denitrification and volatilization), which leave the bioavailable N in soils ($NO_3^-$, $NH_4^+$) enriched in $^{15}N$ (Hogberg, 1997; Robinson, 2001) (Fig. 2-23a). In such ecosystems, plants acquire both higher $\delta^{15}N$ and N content in their leaves, which is characteristic of a more open N cycle. In N-limited ecosystems (i.e., less N availability), by comparison, there is less N-bearing material available for N loss, and hence less opportunity for $^{15}N$ enrichment of the system through such processes. Plants also rely more heavily on mycorrhizal fungi for N acquisition, which is a more $^{15}N$-depleted source (Craine et al., 2009; Hobbie and Hobbie, 2008) (Fig. 2-23b).

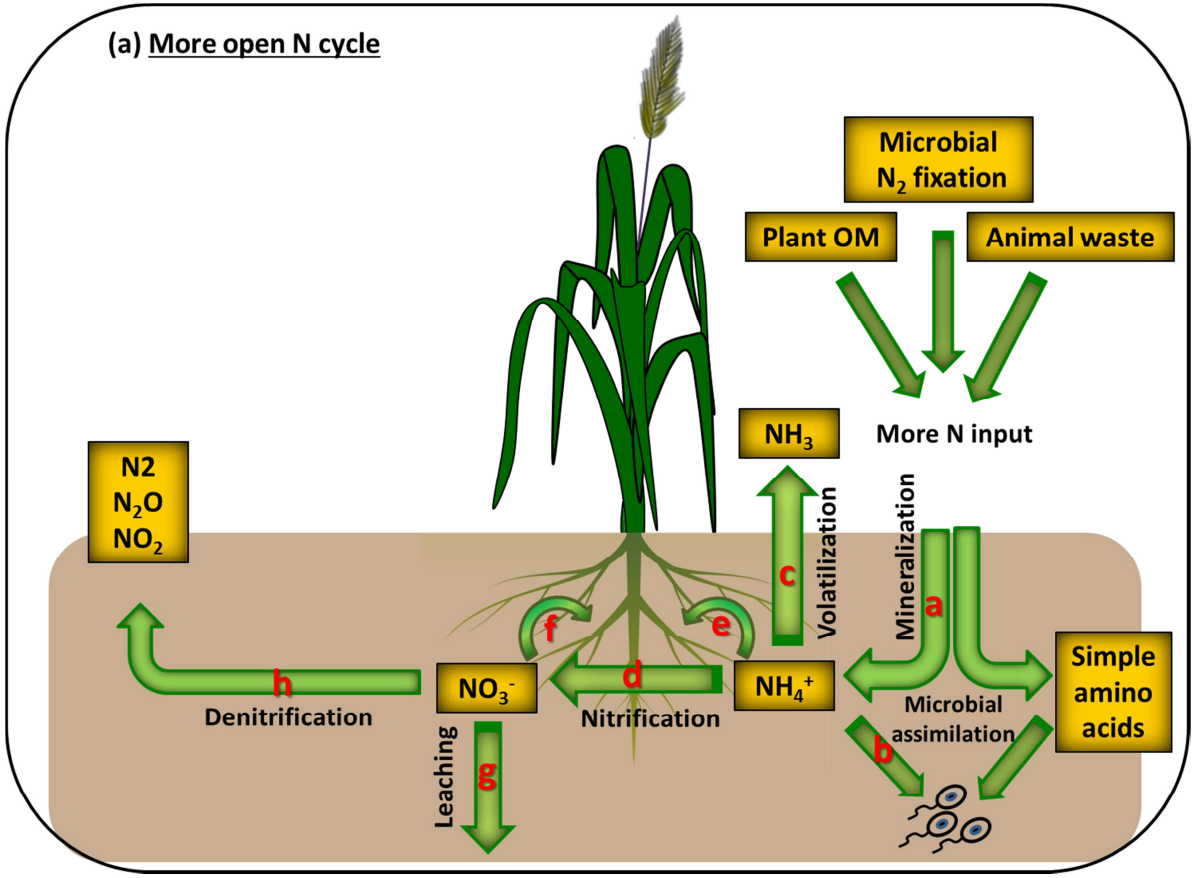


**Figure 2-23a: A simplified model for the "openness" of the N cycle in ecosystems with high N availabilities. (a) N mineralization: Conversion of organic N to $NH_4^+$ (ε = 0-5 ‰); (b) Microbial assimilation: incorporation of $NH_4^+$ into microbial biomass (ε = 14-20 ‰). (c) $NH_3$ volatilization: conversion of $NH_4^+{}_{(aq)}$ to $NH_{3(g)}$ (ε = 40-60 ‰); (d) Nitrification: conversion of $NH_4^+$ to $NO_3^-$ (ε = 15-35 ‰); (e) Plant uptake and assimilation of $NH_4^+$ (ε = 9-18 ‰); (f) Plant uptake and assimilation of $NO_3^-$ (ε = 0-19 ‰); (g) $NO_3^-$ leaching (ε = 0-1 ‰); (h) Denitrification: conversion of $NO_3^-$ to $N_2O$, $N_2$ and $NO_2$ (ε = 28-33 ‰), (ε data from Robinson, 2001 and Houlton and Bai, 2009).**

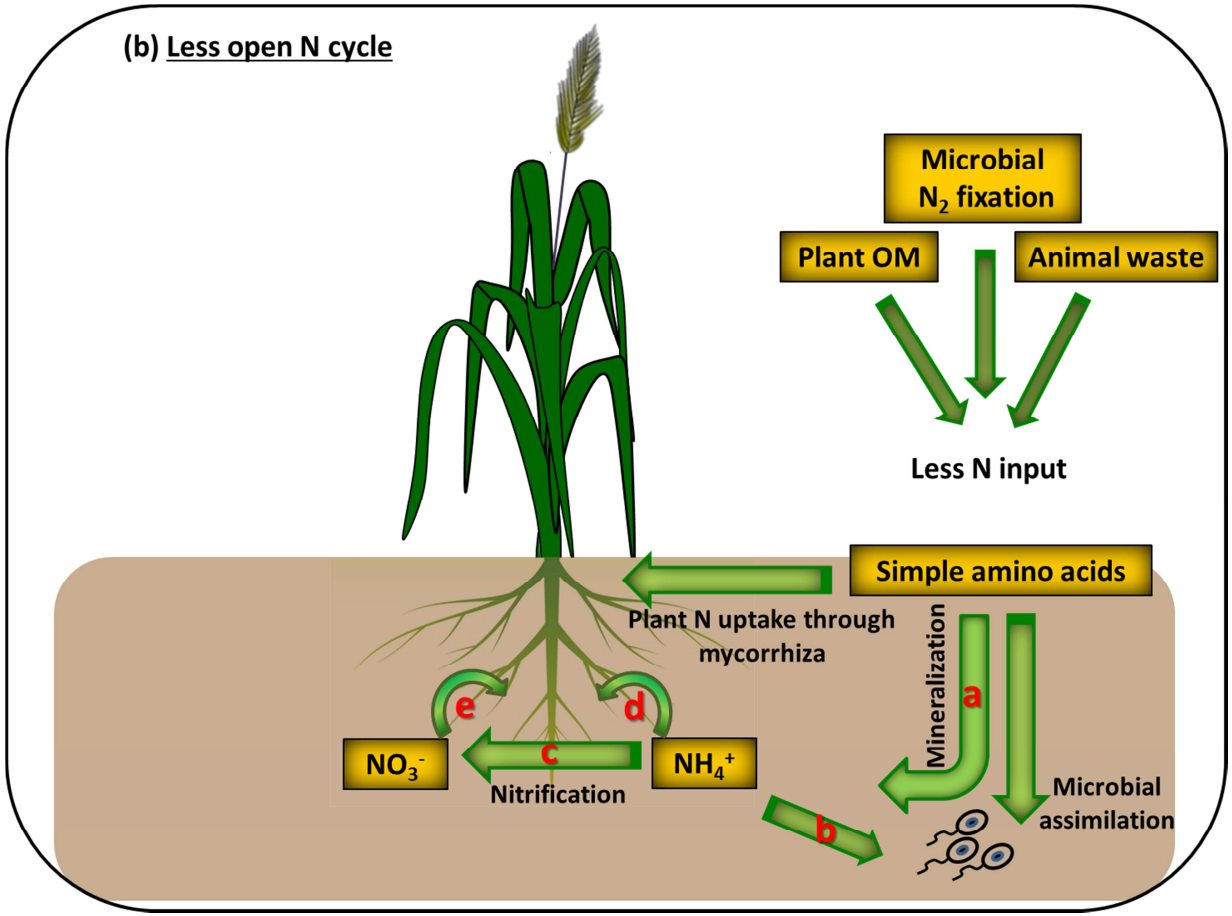


**Figure 2-23b: A simplified model for the "openness" of the N cycle in ecosystems with low N availabilities. (a) N mineralization: Conversion of organic N to $NH_4^+$ (ε = 0-5 ‰); (b) Microbial assimilation: incorporation of $NH_4^+$ into microbial biomass (ε = 14-20 ‰). (c) Nitrification: conversion of $NH_4^+$ to $NO_3^-$ (ε = 15-35 ‰); (d) Plant uptake and assimilation of $NH_4^+$ (ε = 9-18 ‰); (e) Plant uptake and assimilation of $NO_3^-$ (ε = 0-19 ‰), (ε data from Robinson, 2001 and Houlton and Bai, 2009).**

### 2.4.2.3 Spatial differences

The significant difference observed in foliar $\delta^{15}$N among sites S13–3, S13–6 and S13–7 points to the heterogeneity of this ecosystem and the presence of different microhabitats even at small scales. These differences may be related to local variations in soil properties, slope aspects, topography, water availability, animal disturbance and grazing, which in turn could affect local N cycling. While we might predict higher foliar $\delta^{15}$N at S13–7 than S13–6 given the former's higher topsoil OM (9.2 *vs.* 7.5 wt. %) and TN (0.4 *vs*. 0.2 wt. %), the result is opposite (S13-7-avg. –2.0 ‰, n = 6; *vs.* S13-6-avg. +1.4 ‰, n = 8). Some other environmental factors such as slope aspect and topography might overshadow the influence of soil properties. In S13-6, plant samples were collected from the north side of the road on a SW-facing shallow slope, while in S13-7, plants were sampled on south side of the road on a NE-facing, shallow slope. The different slope aspects of these sites likely influence microclimate and vegetation pattern (Sanborn and Jull, 2010), as is indicated by the dominance of subshrubs at S13-7 and herbs at S13-6. Leaves from S13-6 have higher $\delta^{15}$N (avg. +1.4, n = 8) than at S13-3 (avg. –1.3 ‰, n = 11). Soil at site S13-6 probably provides plants with N pools having higher $\delta^{15}$N because of its shallower slope than site S13-3 (Fig. 2-14), as discussed in section 2.4.1.

Plants at S12-5 (avg. –28.7 ‰, n = 7) have a significantly lower average foliar $\delta^{13}$C than at S12-2 (avg. –26.9 ‰, n = 7) and S12-6 (avg. –27.3, n = 7). The adjacency of site S12-5 to a main highway may contribute to lower plants $\delta^{13}$C at this site due to the influence of carbon dioxide generated by vehicle fuel combustion (typically –29.3 to –27.6 ‰; Widory and Javoy, 2003). Site S12-2 is also located adjacent to a main and busy road, but does not have such lowered $\delta^{13}$C plant values. This is most probably due to the sampling position on a steep slope that is well elevated from the road (Fig. 2-13).

### 2.4.2.4 Plant Functional Groups

There is no significant difference in foliar $\delta^{13}$C and $\delta^{15}$N between the herbs and the shrubs/subshrubs. Considering that all plants studied here utilize the $C_3$ photosynthetic pathway, the lack of any clear distinction in foliar $\delta^{13}$C is not surprising. The absence of

systematic differences in $\delta^{15}N$ between these two groupings is less expected, given potential differences in N conservation and resorption efficiency (Bertiller et al., 2005) and patterns of root distribution and rooting depth, which affect resource acquisition (Sala et al., 1989). The absence of a statistically meaningful difference in $\delta^{15}N$ might be due to the unequal sample size of herbs *vs.* shrubs/subshrubs or the effects of many other confounding environmental factors such as plant growth stage, the range of N sources available to all plants, and the wide range of environmental conditions in the study area.

The shrubs and subshrubs, however, have significantly higher C and N contents than the herbs at both foliar and whole plant levels, as has been observed previously, especially for N (Hobbs et al., 1981; Sala et al., 1989). Previous studies have also noted significantly different C, N and other micronutrient contents in soils underlying different plant functional groups (grasses, shrubs and trees) (Gill and Burke, 1999; Kieft et al., 1998; Scholes and Hall, 1996). These features have been attributed to differences in biomass allocation and litter chemistry, with soils under shrubs generally having higher C and N contents than under grasses (Burke et al., 1998; Gill and Burke, 1999; Schimel et al., 1995; Trumbore et al., 1995).

The difference in N content measured here might be related to different N conservation strategies during the late growing season. Arid and semi-arid perennial grasses have lower N contents in senesced leaves and overall higher N conservation efficiency than shrubs, which is an important adaptive trait for plants from nutrient-limited ecosystems (Bertiller et al., 2005). Plants in this study were sampled in very late growing season (mid-late September in 2012 and late August in 2013), with perennial grasses as the main part of herbs group. Hence, the difference in N contents may be explained by higher N resorption efficiency from senescing tissues in grasses than in shrubs.

### 2.4.3 Intra-plant Variations

The $\Delta^{13}C_{\text{other plant part-leaf}}$ spacings measured in this study are positive, consistent with previous studies showing that strongly photosynthesizing tissues (i.e., leaf) have lower $\delta^{13}C$ than other tissues (Brugnoli and Farquhar, 2000; Cernusak et al., 2009; Hobbie and

Werner, 2004; Scartazza et al., 1998). The difference measured here, however, is small (< 1 ‰), and should not affect the use of $\delta^{13}C$ to infer the diet of animals that happen to prefer one plant part over another as forage. In chapter three, we shall examine whether the $\Delta^{13}C_{\text{other plant part-leaf}}$ spacing is sensitive to substantially different atmospheric concentrations of $CO_2$, which could then affect dietary interpretations, both forward and backward in time.

No clear pattern in $\Delta^{15}N_{\text{other plant part-leaf}}$ spacings was observed, likely because of confounding factors including differences in root morphology, depth and distribution, microhabitat, mycorrhizal associations and growing stage. The root crowns examined here, nonetheless, have higher N contents (Fig. 2-15) and $\delta^{15}N$ (Fig. 2-11) than other plant tissues; this difference is significant for N content, but not $\delta^{15}N$. Nitrogen contents of different tissues commonly show significant variations between active growing and senescent stages in herbaceous plants (Bausenwein et al., 2001; Choi et al., 2005; Millard, 1988). This may be related to nutrient conservation, which can include resorption and reallocation of N from leaves into roots during senescence stages (Bertiller et al., 2005). The higher N content of the root crowns relative to other tissues observed in this study may be explained by N allocation from leaves to root crowns for storage late in the growing season. In chapter three, we examine the variations in N content and isotopic composition in these plants under controlled growing conditions, in order to test some of these ideas.

## 2.5 Conclusion

All plants analyzed from this ecosystem follow the $C_3$ photosynthetic pathway, and have an average whole plant $\delta^{13}C$ of –27.5 ± 1.2 ‰ and foliar $\delta^{13}C$ of –28.0 ± 1.3 ‰. This isotopic composition, with suitable correction for the Suess effect (decrease in $\delta^{13}C_{atm}$ resulting from anthropogenic activities, Keeling, 1979 and Verburg, 2006), provides a useful baseline for interpreting the diet of ancient herbivores that lived in this region.

The story for N is more complicated, given the wider range of intra-plant and inter-plant $\delta^{15}N$ variations and the more numerous factors controlling $\delta^{15}N$ of vegetation in different

microhabitats in subarctic ecosystems. Variations in the importance of N availability and water availability in controlling the $\delta^{15}N$ of plants among a range of local microhabitats, and possible fluctuations in these factors in the past, limits our ability to use a modern N isotopic baseline for vegetation for paleodietary models in these ecosystems. Nonetheless, these data for modern plants provide a good representation of the nature of present N cycling in this region. Its comparison with equivalent data for ancient plants from Beringia could reveal any changes in the nature of N cycling in this ecosystem from Pleistocene to present time (Chapter 3).

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# Chapter 3

# 3 The Eastern Beringian Nitrogen Cycle before and after the Terminal Pleistocene

## 3.1 Introduction

During the most recent glacial-interglacial cycle (18k to 10k $^{14}$C a BP) (Fox-Dobbs et al., 2008), a succession of several environmental changes (climatic, atmospheric and biotic) caused a dramatic shift from the face of late Pleistocene Beringia to what we see today in the same region. During the late Pleistocene, between 50-10k $^{14}$C a BP, a widespread extinction of mammals with large bodies (≥ 44 kg) occurred everywhere except Africa (Barnosky et al., 2004; Koch and Barnosky, 2006). In North America 35 genera of large mammals were lost (Faith and Surovell, 2009), with the majority (29 genera) disappearing globally and only 6 genera surviving elsewhere (Faith and Surovell, 2009). The exact timing of this extinction and extirpation is debated (Faith and Surovell, 2009; Fiedel, 2009; Fiedel and Haynes, 2004; Grayson, 2001, 2007; Grayson and Meltzer, 2003; Zazula et al., 2014), but almost all of them were lost permanently after the terminal Pleistocene (~12-10k $^{14}$C a BP) (Faith and Surovell, 2009).

Coincident with the extinction of these large mammals (Gill et al., 2009; MacDonald et al., 2012; Mann et al., 2013), North America also experienced a dramatic change in climate (Fritz et al., 2012; Williams et al., 2001), size of ice sheets (Stuart, 1991) and range and composition of vegetation (Gill et al., 2009; Williams et al., 2001), all of which was accompanied by a large global rise in atmospheric $CO_2$ concentration ($pCO_2$) (Marino et al., 1992; Martinez-Garcia et al., 2014; Parrenin et al., 2013; Petit et al., 1999). The significant changes in soil, plant and animal communities at this time made a great alteration in the face of Beringia with no exact modern analogue (Gill et al., 2009; Höfle et al., 2000; Williams et al., 2001). Given the strong feedback mechanisms among herbivores, plant nutrient content and ecosystem nutrient cycling that govern the function of modern ecosystems (Pastor et al., 2006), a comparable shift in nutrient cycling with such major environmental changes is expected in Beringia.

Faith (2011) has suggested a mode transition in N cycling as the main cause of megafauna extinction in North America after the terminal Pleistocene, which was driven mainly by the change in the N content of plants. A global study of lake sediment nitrogen isotopic compositions ($\delta^{15}$N) from a wide range of ecosystems also reported a gradual decrease in N availability of terrestrial ecosystems between ~15-7 ka, which suggests a shift in the nature of terrestrial N cycling (McLauchlan et al., 2013). A change in the nature of N cycling could be tracked using $\delta^{15}$N of plants and animals. Higher plant $\delta^{15}$N reflect higher N availability and a more open N cycle (Hietz et al., 2011; Hogberg, 1997; Pardo et al., 2006). This higher $\delta^{15}$N is passed on to the second trophic level (consumers) through the food chain (DeNiro and Epstein, 1981; DeNiro, 1985). Several studies have reported significantly different $\delta^{15}$N for herbivores over different time periods (pre-, full- and post-LGM) in Alaska (Fox-Dobbs et al., 2008) and Eurasia (Iacumin et al., 2000; Richards and Hedges, 2003; Stevens and Hedges, 2004; Stevens et al., 2008), and related those differences to a possible shift in $\delta^{15}$N of their diet in response to climate change (temperature and precipitation). This could indirectly point to a shift in N cycling in these ecosystems. Considering these studies and empirical evidence about the influence of environmental factors on terrestrial N dynamics (Billings et al., 2004; Craine et al., 2009; Frank et al., 2000; Hungate et al., 1997; Schulze et al., 1991; Williams et al., 2006; Wolf et al., 2010), a possible shift in N cycling should be expected in response to the late Pleistocene environmental change. If this is true, then a different N isotopic baseline should be utilized for the late Pleistocene ecosystems when interpreting and comparing $\delta^{15}$N of fossil bones with that of their modern counterparts (Szpak et al., 2013).

The purpose of this study is to use the stable isotope compositions of plants and animals to test for possible changes in N cycling in eastern Beringia between the late Pleistocene and present time. Such changes may have accompanied the massive megafauna extinction and environmental changes at the terminal Pleistocene. An archive of the late Pleistocene Beringian flora can be found in fossil nests of arctic ground squirrels (*Spermophilus parryii*) and trapped in perennially frozen sediments. In this study, we have:

- Analyzed $\delta^{13}C$ (carbon isotopic composition) and $\delta^{15}N$ of plant macrofossils and fossil bones (collagen and structural carbonate) recovered from these nests, which were collected from placer goldmines in the west central Yukon Territory, to define a C and N isotopic baseline for the late Pleistocene eastern Beringia;

- Performed for comparison the same analysis of modern plants and arctic ground squirrel bones (collagen and structural carbonate) from the eastern shoreline of Kluane Lake and the south central Whitehorse valley;

- Used the N and C isotopic fractionation between the modern squirrel bone collagen and plants to estimate how well the late Pleistocene Beringian plant macrofossils had preserved their original isotopic compositions;

- Measured the oxygen ($\delta^{18}O$) and hydrogen ($\delta^{2}H$) isotopic compositions of Pleistocene water from some frozen nests and two modern water sources in the study areas to evaluate whether the $\delta^{18}O$ of bioapatite structural carbonate from ground squirrel bone was reflective of original environmental water, and

- Monitored the $\delta^{13}C$ and $\delta^{15}N$ of six species representing the most common Beringian plants, over 317 days during two different treatments: 'not buried' and 'buried' under a thin layer of loess soil to test for isotopic changes in plant macrofossils that could result from microbial and/or oxidative decomposition.

### 3.1.1 Terrestrial N Cycling and $\delta^{15}N$ of Soils and Plants

In terrestrial ecosystems, N enters into the soil through either biotic reactions (decomposition of organic matter (OM) and $N_2$ fixation by microorganisms) or abiotic N deposition from atmosphere (lighting and rainfall). Once in the soil, such N ($NH_4^+$) could undergo nitrification to $NO_2^-$ and $NO_3^-$, or denitrification to $N_2$, $N_2O$ and NO (Fig. 3-1). The importance of different processes in the N cycle is ecosystem-specific, and highly affected by climatic and environmental factors (Havlin et al., 2005). Both $NO_3^-$ and $NH_4^+$ are bioavailable for plants. In addition to inorganic forms of N, dissolved organic

nitrogen (DON) also is an important source of N for plants (Kielland et al., 2006; Mobley et al., 2014; Näsholm et al., 2009; Weigelt et al., 2004). Given the complexity of N cycle arising from different rates of these reactions in different ecosystems and the different N isotopic fractionations ($\varepsilon$) of these reactions (Hogberg, 1997; Robinson, 2001), there are differences in $\delta^{15}$N among soil bioavailable forms of N. Bulk soil $\delta^{15}$N, therefore, is not always a good representation of source N for plants because it might be dominated by non-bioavailable N (Hogberg, 1997).

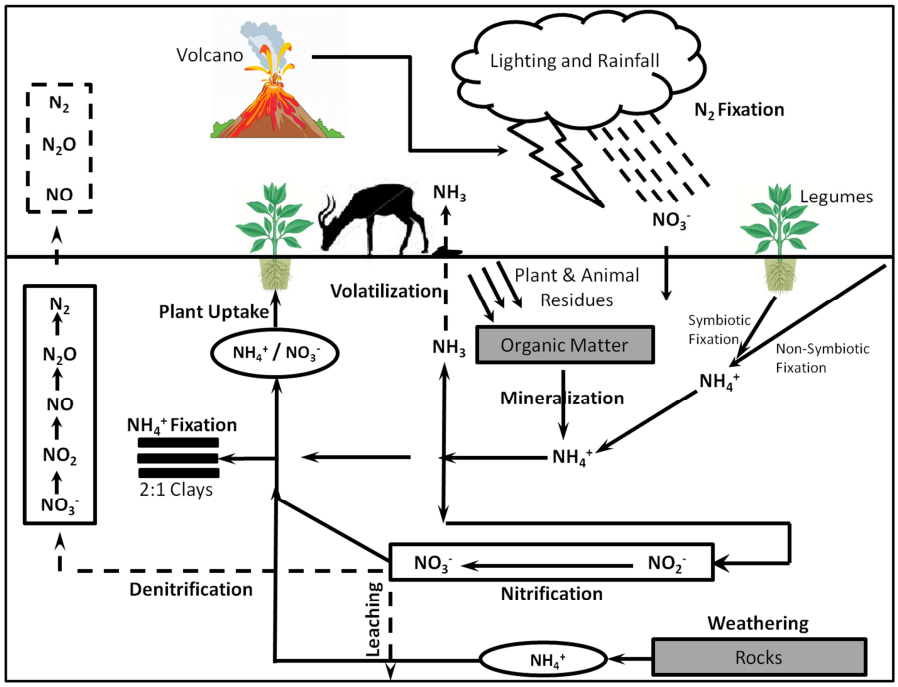


**Figure 3-1: N cycling in terrestrial ecosystems; solid lines indicate main input and internal N reactions and dashed lines indicate main N loss reactions (adapted from Havlin et al., 2005).**

The ratio of the rate of N loss processes (denitrification, volatilization and leaching) to internal N cycling (nitrification, plant uptake, mineralization and immobilization) determines the openness of the N cycle (Reichmann et al., 2013). A higher rate of N loss processes relative to internal N cycling produces a more open N cycle. A more open N cycle can be reflected in higher $\delta^{15}$N of plants (Hietz et al., 2011; Hogberg, 1997; Martinelli et al., 1999; McLauchlan et al., 2007; McLauchlan et al., 2013; Pardo et al.,

2006). This behaviour arises from an increased N supply for N loss reactions, which leaves the soil system highly enriched in $^{15}N$ due to the large $\varepsilon$ between inorganic N in the liquid phase and its evolved gaseous forms (Hogberg, 1997; Robinson, 2001). In fact, plant $\delta^{15}N$ has potential as an index of ecosystem N availability (Craine et al., 2009) and openness of the N cycle (Hietz et al., 2011). Tropical forests with higher N availability than temperate forests have relatively higher rates of N loss reactions and therefore present higher plant $\delta^{15}N$ and a more open N cycle (Martinelli et al., 1999). Long-term changes in foliar N content and $\delta^{15}N$ in tropical forests also has been attributed to a shift in N cycling (Hietz et al., 2011). Several studies also connected higher plant $\delta^{15}N$ in drier sites with a more open N cycle than at wetter sites (Austin and Vitousek, 1998; Reichmann et al., 2013).

### 3.1.2 Plants and Soils $\delta^{13}C$

Plant $\delta^{13}C$ is a function of the photosynthesis pathway through which atmospheric $CO_2$ is fixed. Among vascular plants, $C_3$ plants are characterized by values ranging from –38 to –22 ‰. $C_4$ plants have higher values (–21 to –9 ‰) and CAM plants have intermediate values (–30 to –13 ‰) (Tieszen, 1991; Yeh and Wang, 2001). Most modern plants in west central Yukon Territory are $C_3$ (Welsh, 1974; Wooller et al., 2007); $C_4$ plants are rarely found in this area (Sage et al., 1998; Wooller et al. 2007). Carbon isotopic variation in $C_3$ plants is controlled by factors including source $CO_2$ $\delta^{13}C$, $pCO_2$, water availability, latitude, altitude and irradiation (Korner et al., 1991; Polley et al., 1993; Szpak et al., 2013; Tieszen, 1991). Water availability appears to be the most important factor (Murphy and Bowman, 2009). In a C isotopic study of modern grasses and sedges from Alaska and Yukon Territory, significantly higher $\delta^{13}C$ were reported for plants from dry habitats than those from wet habitats (Wooller et al., 2007). Plants respond to water stress and aridity through stomatal closure which then results in reduced discrimination against $^{13}C$, higher water use efficiency and less negative $\delta^{13}C$ (Tieszen, 1991).

The influence of $pCO_2$ and $\delta^{13}C$ of atmospheric $CO_2$ ($\delta^{13}C_{atm}$) on plant $\delta^{13}C$ was defined by Farquhar et al. (1989):

Equation 3.1 $$\delta^{13}C_{plant} = \delta^{13}C_{atm} - a - (b - a) \times (C_i/C_a)$$

where *a* is diffusion fractionation of $^{13}C$ between ambient and intercellular $CO_2$ (+4.4 ‰), *b* is net fractionation by RuBisCO carboxylation (ca. +28 ‰), and $C_i$ and $C_a$ are intercellular and ambient $pCO_2$, respectively. Given these controls, a decrease in stomatal density and plant $\delta^{13}C$ during deglaciation (15-12 ka), when $pCO_2$ increased from 190 to 280 ppm, has been reported for fossil limber pine needles from rat middens from Western United States (Van de Water et al., 1994). This effect may be farther amplified since the Industrial Revolution (~1850 AD), given the additional increase in $pCO_2$ accompanied by a decrease in $\delta^{13}C_{atm}$ resulting from anthropogenic activities (known as the Suess effect) (Keeling, 1979; Verburg, 2006). It is important to take such factors into account when comparing $\delta^{13}C$ of modern and late Pleistocene fossil plants and bones.

Vegetation following different photosynthesis pathways ($C_3$, $C_4$ and CAM) impart different $\delta^{13}C$ signals to their underlying soils, given that plants are a major source of organic carbon (OC) to soil (Andreeva et al., 2013). Stevenson et al. (2005) suggested that even small changes in $\delta^{13}C$ of plant communities are transmitted to soil OC. Therefore, the $\delta^{13}C$ of soil OC has been used to track vegetation changes in a range of ecosystems (Aucour et al., 1999; Francisquini et al., 2014; Freitas et al., 2001). In transferring $\delta^{13}C$ signatures of plants to soils, however, decomposition may cause some deviation of soil OC-$\delta^{13}C$ from original signals, which is discussed more fully in section 3.1.4.

### 3.1.3 N, C and O Isotopic Compositions of Bone

Stable C and N isotopic analyses are useful tools for reconstructing the diet (Bocherens, 2003; Bocherens et al., 2014; Koch et al., 1995; Tiunov and Kirillova, 2010) and trophic positions of animals in food webs (Ambrose, 1991). The main hypothesis behind the use of these analyses in trophic ecology is presented best in the statement that “You are what you eat plus a few per mil” (DeNiro and Epstein, 1978, 1981). In fact, it is suggested that consumers’ isotopic compositions can resemble the isotopic signatures of their diet (primary producers) in food webs. Determining those “… few per mil” (stepwise isotopic

enrichments) and identifying the factors affecting this difference for both C and N, however, has been the subject of many studies (e.g. Ambrose, 1991; Boecklen et al., 2011; DeNiro and Epstein, 1978; Kelly and Martínez del Rio, 2010; Kurle et al., 2014; McClelland and Montoya, 2002). A wide range of factors affect the isotopic composition of primary producers (e.g. photosynthesis pathway, climate) and consumers (e.g. age, gender, tissue type, body size, reproductive status), and the trophic discrimination between consumers and producers (e.g. macromolecular content of diet, consumer physiology, ecosystem). Accordingly, it is important to define an appropriate local C and N isotopic baseline and the isotopic tissue separations between trophic levels in any application of stable isotope analysis in trophic ecology of food webs (Ambrose, 1991).

Bone collagen is one of the most common tissues analyzed in trophic ecology and paleodietary reconstruction because: (i) it is the most abundant and one of the most stable proteins, and is commonly well-preserved in animal macrofossils, and (ii) it reflects an isotopic average of an animal's diet over several years before death (Bocherens et al., 2014). The N isotopic separation between collagen and diet ($\Delta^{15}N_{Col\text{-}diet}$) follows the same trend as for whole organism and diet (~2-4 ‰ enrichment) (Koch et al. 1994). In contrast, C isotopic separation between collagen and diet ($\Delta^{13}C_{Col\text{-}diet}$) is different from that of whole animal and diet (~1-5 ‰ *vs.* ~1 ‰) (Koch et al. 1994). These enrichments, however, can be quite variable. The most widely observed range for $\Delta^{13}C_{Col\text{-}diet}$ and $\Delta^{15}N_{Col\text{-}diet}$ from different experimental conditions is +3 to +6 ‰ (Lee-Thorp et al., 1989; Van Der Merwe, 1982) and +2 to +4 ‰ (e.g. Ambrose, 2000; Bocherens and Drucker, 2003; Hare et al., 1991; Koch et al. 1994), respectively. Ambrose and Norr (1993) and Tieszen and Fagre (1993) have reported a systematic change from –2 to +10 ‰ in C isotopic separation between collagen (which reflects dietary protein) and diet with changing $\delta^{13}C$ of dietary protein. Variable $^{15}N$ enrichment from bulk diet to bone collagen also have been reported for different climates, ecosystems and animal physiologies, with larger $^{15}N$ enrichments commonly reported for hotter and drier conditions relative to colder and wetter environments (Ambrose, 1991; Ambrose, 2000; Hilderbrand et al., 1996).

The isotopic composition of bioapatite from bone (and tooth enamel) also has a large application in dietary reconstruction. Biopatite-structural carbonate is incorporated from blood bicarbonate, which is more reflective of whole diet $\delta^{13}C$. Bioapatite-structural carbonate, however, is more enriched in $^{13}C$ than collagen relative to whole diet. A $^{13}C$ enrichment from diet to structural carbonate of +9 to +11 ‰ has been reported for rodents (Ambrose and Norr, 1993; DeNiro and Epstein, 1978; Jim et al., 2004; Tieszen and Fagre, 1993) and +12 to +14 ‰ for herbivorous ungulates (Balasse, 2002; Cerling and Harris, 1999; Passey et al., 2005; Sullivan and Krueger, 1981; Thorp and Van Der Merwe, 1987). Krueger and Sullivan (1984) suggested that the C isotopic spacing between structural carbonate and collagen ($\Delta^{13}C_{Sc\text{-}Col}$) decreases with increasing trophic level status (herbivores, omnivores and carnivores), and Lee-Thorpe et al. (1989) have reported a mean $\Delta^{13}C_{Sc\text{-}Col}$ of +6 to +7 ‰ for both captive and wild herbivores, +5 ‰ for omnivores and +4 ‰ for carnivores. This change may reflect different proportions in the macromolecular compositions (carbohydrate, lipid and protein) of diet among animals at different trophic levels and/or different digestive physiologies of animals at different trophic levels (Crowley et al., 2010; Hedges, 2003).

Here we have determined the enrichment in $^{13}C$ and $^{15}N$ from modern bulk plants to modern ground squirrel bone collagen for the Kluane Lake and Whitehorse areas, and applied it to that of late Pleistocene Beringia to examine possible change in original isotopic compositions of fossil plants. The $\Delta^{13}C_{Sc\text{-}Col}$ was used to identify the trophic position of both the modern ground squirrels and ancient rodents that were sampled.

The $\delta^{18}O$ of bioapatite structural carbonate ($\delta^{18}O_{Sc}$) in mammals reflects their drinking water ($\delta^{18}O_{dw}$), the isotopic composition of which is typically close to local meteoric water (Longinelli, 1984). The $\delta^{18}O$ of meteoric water is determined by the source, temperature, humidity, latitude and fractionation processes during water phase transformations (Dansgaard, 1964; Fricke and O'Neil, 1999; Rozanski et al., 1993). Therefore, $\delta^{18}O_{Sc}$ of water-dependent mammals, if well preserved after death, has the potential to record the $\delta^{18}O$ of meteoric water and hence the climatic conditions whilst they were living. In the present study, we have combined the relationship between $\delta^{18}O_{Sc}$

and $\delta^{18}O$ of $PO_4$-bioapatite ($\delta^{18}O_P$) measured by Iacumin et al. (1996) for a variety of terrestrial mammals (Equation 3.2) with the relationship between $\delta^{18}O_P$ and $\delta^{18}O_{dw}$ (Equation 3.3) reported by Luz and Kolodny (1985) for rodents to obtain an estimate of meteoric water $\delta^{18}O$ in three main study areas (Klondike, Kluane Lake and Whitehorse):

Equation 3.2 $$\delta^{18}O_P = 0.98\ (\delta^{18}O_{Sc}) - 8.5$$

Equation 3.3 $$\delta^{18}O_{dw} = (\delta^{18}O_P - 17.88)/0.49$$

### 3.1.4 Decomposition of Plant Materials

Decomposition is an essential part of nutrient cycling and functioning of terrestrial ecosystems (Veen and Kuikman, 1990; Wang et al., 2004). It provides C and energy for heterotrophic microbes and releases nutrients for both plants and soil microorganisms. Decomposition is a prolonged transformation of organic compounds through a series of sequential biological processes from one form into another form at different rates (Veen and Kuikman, 1990). There are different phases of decomposition with different rates of change in the quality of plant materials, composition of microbial communities, and nature of microbial metabolisms, all of which affect the elemental and isotopic compositions of decomposed plants.

#### 3.1.4.1 Factors Affecting Plant Decomposition

The rate of plant litter decomposition is highly dependent on: (i) the chemical composition of plant materials (C/N, and N, lignin and polyphenol contents) (Singh and Gupta, 1977; Wang et al., 2004), (ii) environmental conditions of decomposition (soil texture, pH, oxygen, moisture content, temperature), and (iii) soil fauna (microorganism communities and soil animals) (Singh and Gupta, 1977).

The amount of water-soluble compounds and N content of plant materials largely controls the rate of OM decomposition in early stages, while lignin and polyphenol contents of OM control the rate during the late stages of decomposition (Melin, 1930; Wang et al., 2004). In general, the initial chemical composition of plant materials is an important factor affecting their rate of decomposition (Wang et al., 2004), and the

composition of the microbial communities involved (Aneja et al., 2006; Blagodatskaya and Anderson, 1998; Ha et al., 2007).

The growth and activity of soil bacteria and fungi, the primary decomposers of soil OM, are largely controlled by soil pH, temperature and availability of water and oxygen. Under acidic conditions fungal respiration increases while bacterial activity decreases (Bewley and Parkinson, 1985; Blagodatskaya and Anderson, 1998). Batty and Younger (2007) have shown that the pH of substrate does not significantly affect the rate of decomposition in wetlands, but can affect nutrient bioavailability for plants and microorganisms. The influences of soil temperature and water content on decomposition are interrelated (Bunnell and Tait, 1974; Campbell and Biederbeck, 1976). Moisture availability has been reported to be even more important than temperature in driving microbially mediated decomposition (Campbell and Biederbeck, 1976). Increasing temperature can promote litter decomposition more readily in well-drained moist tundra with sufficient oxygen for aerobic activities than in saturated wet soils dominated by anaerobic activities (Mikan et al., 2002).

Increasing temperature between 0 and 25°C is known to increase respired $CO_2$ from soil decomposition in Arctic ecosystems (Flanagan and Veum, 1974; Heal and French, 1974) but this response is non-linear and site-specific (Oberbauer et al., 1996). For a long time, it was assumed that microbial activity and respiration ceased under frozen conditions. Recent studies, however, have shown that significant levels of microbial activities occur in the Arctic even during frozen conditions (Clein and Schimel, 1995; Jones et al., 1999; Price and Sowers, 2004; Welker et al., 2000). Microbial activity decreases at lower temperatures, but does not cease (Mikan et al., 2002). This is particularly important in C and N cycling within Arctic ecosystems (Welker et al., 2000) and could explain much of the C loss during the long, cold seasons (Hobbie and Chapin, 1996; Moore, 1983). The presence of unfrozen water films around soil particles at temperatures as low as –20 to –40°C allows diffusion of microbial substrates and waste products (Price and Sowers, 2004). As long as there is small amount of liquid water in soils, microbes can be active (Mikan et al., 2002; Rivkina et al., 2000).

There is still no full understanding of the temperature-dependent responses of Arctic microbes. Some studies report a strong response to temperature (Clein and Schimel, 1995; Flanagan and Veum, 1974; Hobbie and Chapin, 1996), while others suggest that litter quality is most important during normal summer temperatures (Nadelhoffer et al., 1991; Schmidt et al., 1999). Bosatta and Agren (1999) have hypothesized that the decay of high quality substrates (more labile OM) is less temperature dependent than low quality substrates (recalcitrant OM) and Mikan et al. (2002) have shown this dependency in thawed but not frozen soils. This idea is based on the thermodynamic argument that enzymes involved in metabolizing complex and aromatic substrates require higher activation energies and are more temperature dependent than enzymes metabolizing simple molecules. Schimel and Mikan (2005) have suggested a seasonal shift in microbial substrate use in Arctic soils from N-poor detritus during the growing season to N-rich microbial products during winter, with concomitant seasonal control on N transformation (summer N immobilization *vs.* winter N mineralization).

Soil texture is another important control on decay of OM (Veen and Kuikman, 1990; Gaillard et al., 1999). According to Veen and Kuikman (1990), soil texture can determine the accessibility of OM to microbes by controlling water flow and microbial movement, and facilitating entrapment of OM on mineral particle surfaces, which likely affects microbial turnover processes (uptake, intercellular transformation and release of OM). Soil also plays a significant role in providing nutrients, absorbing undesirable metabolic products, buffering pH, and balancing water and oxygen availability for microbes (Herman et al., 1976).

Soil fauna can also facilitate the process of decomposition (Singh and Gupta, 1977). Earthworms feeding on plant debris, for example, can pass these materials through their gut, change the nutrient content, pH and surface area of the vegetal detritus and make them more easily accessible for microbially mediated decay (Spiers et al., 1986). Burrowing and casting activities of earthworms can also significantly increase the porosity and aggregation of soils, thus affecting the water and oxygen balance in soil micropores (Domínguez et al., 2004). Given the larger range of movement of

earthworms, they can also benefit microflora and microfauna (including fungi and bacteria) with limited ability to move by eating, carrying and excreting them along soil profiles (Brown, 1995).

Earthworms are found in all ecosystems except deserts and perennially frozen soils. Most earthworms are freeze-intolerant organisms (Holmstrup and Zachariassen, 1996; Lee, 1985). Some freeze-tolerant species (like *Eisenia Nordenskioeldi*), however, are present in cold boreal zones and permafrost regions (Holmstrup et al., 1999; Holmstrup and Zachariassen, 1996). The expansion of Wisconsinan glaciers likely caused the extirpation of native North America earthworms in Beringia (James, 2004). Since earthworms have very soft tissues that undergo rapid decomposition immediately after death, finding fossil earthworms to confirm their presence in the past is almost impossible (Schwert, 1979). Some fossil earthworm burrows of presumed Pleistocene age, however, have been described in Europe by Wilcke (1960). The only earthworm-related fossil found in North America (from a postglacial lacustrine sequence in southern Ontario) is a complete earthworm cocoon (the case in which earthworm embryos are developed) with approximate age of 10k $^{14}$C a BP (Schwert, 1979). The lack of physical evidence for earthworms in Beringia, however, should not be used to reject their possible presence and contribution to nutrient cycling during late Pleistocene interglacial periods.

#### 3.1.4.2 $\delta^{15}$N and $\delta^{13}$C Shifts during Plant Decomposition

Several studies have reported enrichment of decomposed plants in $^{13}$C and $^{15}$N such that soil OM has higher $\delta^{13}$C and $\delta^{15}$N than fresh plant tissue inputs (Lichtfouse et al., 1995; Freudenthal et al., 2001; Kramer et al., 2003; Krull et al., 2002; Shearer and Kohl, 1986; Tremblay and Benner, 2006; Wang et al., 2008; Wynn et al., 2006; Xu et al., 2010). Most of our knowledge of how microbial decomposition can change the $\delta^{13}$C and $\delta^{15}$N of plant tissues comes from studying soil OM.

The significant enrichment of soil OM in $^{13}$C and $^{15}$N relative to fresh litter inputs has been attributed to microbial decomposition (Chen et al., 2002; Krull et al., 2002), but the processes are not fully understood. One possible process, preferential decomposition of

$^{13}C$ and $^{15}N$-depleted compounds, is not yet supported by convincing evidence (Lerch et al., 2010). This process would require preservation of $^{15}N$- and $^{13}C$-enriched compounds in soil, which is unlikely because $^{13}C$-enriched compounds such as cellulose, starch and sugars are highly mobile and used by microbes in early stages of decomposition (Benner et al., 1987; Minderman, 1968).

A second possibility involves kinetic isotopic fractionation associated with microbial respiration for C (Nadelhoffer and Fry, 1988; Schweizer et al., 1999) and microbial metabolism for N (Dijkstra et al., 2006). This model produces microbial biomass that is enriched in $^{13}C$ and $^{15}N$, and contributes these products to residual OM (Dijkstra et al., 2006). A role for this process is supported by some studies reporting highly $^{13}C$-depleted evolved $CO_2$ during decomposition (Fernandez and Cadisch, 2003; Schweizer et al., 1999), while such $CO_2$ is not observed in other studies (Boström et al., 2007; Ekblad and Högberg, 2000; Ekblad et al., 2002) or is not accompanied by $^{13}C$-enriched microbial biomass (Blair et al., 1985; Schweizer et al., 1999).

Notwithstanding this debate, contribution of $^{13}C$-enriched, microorganism-related molecules to decomposing OM has been noted in many studies. Microorganisms have higher $\delta^{13}C$ than fresh plant materials (by 1-3 ‰) (Dijkstra et al., 2006; Lerch et al., 2010), both for fungi (Gebauer and Taylor, 1999; Gleixner et al., 1993; Kohzu et al., 1999; Taylor et al., 2003; Wallander et al., 2004) and bacteria (Macko and Estep, 1984). This idea is supported by decomposition studies that report decreasing C/N for OM that approach those of microbes (Wallander et al., 2003), and increasing abundances of microbially derived compounds with increasing soil depth (Huang et al., 1996). Enrichment in $^{15}N$ of decomposed OM also has been explained by the higher $\delta^{15}N$ of microbial products than fresh plant tissues. A significant, metabolism-related, positive trophic shift in $^{15}N$ has been reported for soil microorganisms (ranging from 1.5 to 6 ‰) (Dijkstra et al., 2006; Lerch et al., 2010; Pörtl et al., 2007).

Along with the change in isotopic compositions of decomposed plants, a change in elemental composition has also been observed. Preferential loss of carbohydrates during early stages, and selectively preservation of aromatic compounds (lignin) during late

stages of decomposition have been reported for both woody plants and grasses (Benner et al., 1987; Hedges et al., 1985; Spiker and Hatcher, 1987). Generally, a decrease in total mass and C/N (Benner et al., 1990; Cahyani et al., 2002; Connina et al., 2001; Salazar et al., 2012; Tremblay and Benner, 2006), and increase in N content (Benner et al., 1990; Connina et al., 2001; Salazar et al., 2012; Tremblay and Benner, 2006) of plant detritus and soil OM (Boström et al., 2007), has been observed in most decomposition studies under both aerobic and anaerobic conditions.

Arctic ground squirrel nests, which are the focus of this study, are a mixture of relatively fresh plant tissues and fungal hyphae, bacteria colonies, seeds, spores, hairs, insects and fauna remains and hence are decomposed more quickly than soil OM because of their higher content of fresh OC. The nests can be considered as a temporal hot spot for microbial acquisition of C and energy. The environment surrounding these nests likely controls the rate of microbially mediated decay. There are three potential time periods during which these plant tissues may be subject to decomposition: (i) on the ground surface after senescence but prior to collection by squirrels, (ii) after collection and storage in their nests, and (iii) following burial in perennially frozen sediments. In this study, the $\delta^{13}$C and $\delta^{15}$N of tissues from six different plant species over 317 days (end of one growing season (October 21, 2013) to almost the end of next growing season (September 2, 2014)) have been measured to examine the changes that occur during the first two time periods. The perennially frozen conditions of the third potential time period likely limit decomposition of plant tissues, but cannot halt it completely, given the activities of sub-zero adapted microbes.

## 3.2 Study Area: Placer Goldmines of West-central Yukon Territory

The Klondike area of west-central Yukon Territory, Canada, is located on the eastern side of Beringia (Kotler and Burn, 2000). Fraser and Burn (1997) suggested a grassland cover for this area (after 27k $^{14}$C a BP to the end of Pleistocene) similar to that of Kluane Lake shoreline. The placer goldmines of the Klondike contain perennially frozen ice- and organic-rich loess deposits named “muck” by placer miners; this “muck” provides a

window into the paleoenvironment record of the late Pleistocene (Zazula et al., 2005). The majority of loess in this area was deposited after 27k $^{14}$C a BP followed by accumulation of organic material on top of the silt in the early Holocene (Fraser and Burn, 1997; Kotler and Burn, 2000). The loess was derived most probably from floodplain of Yukon River and Klondike River during late Pleistocene dry periods when the flow rate was low (Fraser and Burn, 1997). The presence of mummified carcasses of various fauna in the frozen "muck" indicates that perennially frozen conditions began shortly after death and deposition (Kotler and Burn, 2000).

Presently, these perennially frozen sediments occur mostly on north and north-easterly facing slopes, but are absent from south-facing slopes that are now occupied by deciduous woodlands (Kotler and Burn, 2000; Zazula et al., 2005). A mean annual temperature of –1.7ºC has been measured at the top of permafrost in undisturbed ground in Dawson area (Smith et al., 1998). Within the immediate study area, fossil arctic ground squirrel nests were recovered at exposures along Quartz Creek (QC), Independence Creek (IC), Sulphur Creek (SC), Eureka Creek (EC) (Fig. 3-2). A few samples were obtained from placer goldmines located in Glacier Creek (GC) in Sixty mile River area and Little Blanche Creek (Fig. 3-2).

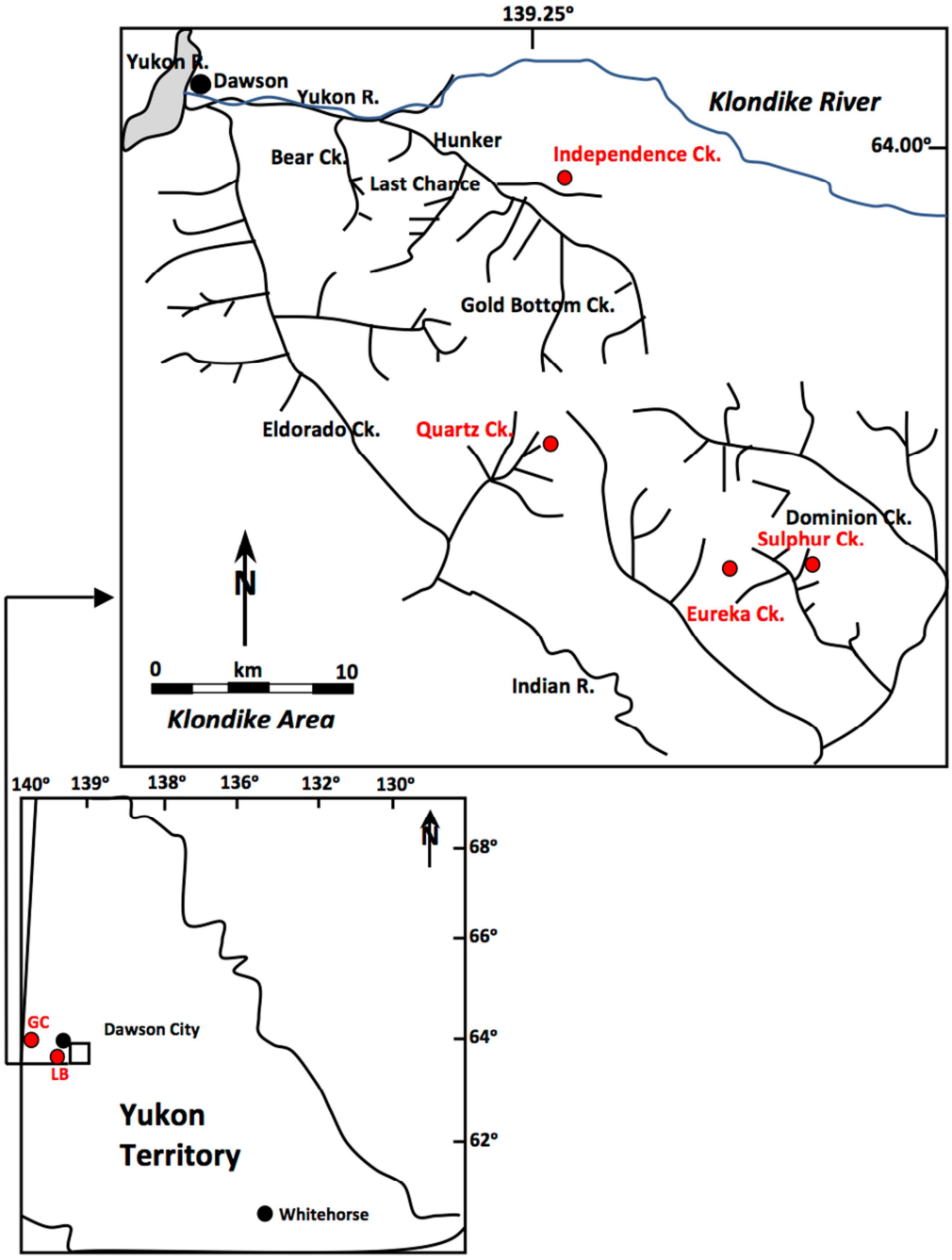


**Figure 3-2: Location of sampling sites (After Kotler and Burn, 2000).**

## 3.3 Materials and Methods

### 3.3.1 Design of Decomposition Experiment

In October 2013, six wooden boxes (17 cm (width) × 28 cm (length) × 8 cm (depth)) were prepared and divided in half using a layer of polystyrene (Fig. 3-3a). The bottom of each box was covered with a layer of loess paleosol (2 cm thickness) collected from Quartz Creek (QC-7). Air-dried stems, leaves and inflorescence of six modern plant species (*Poa glauca, Elymus trachycaulus, Artemisia frigida, Calamogrostis purpurascens, Festuca altaica, Elymus spicatus*) collected from eastern Kluane Lake were cut into ~2-3 cm-long pieces. These species were most probably among the most common plants in eastern Beringia during the late Pleistocene (Zazula et al., 2006b, 2007). A layer of cut plant tissue (2 cm thick) from each species was placed on top of the soil layer in each box. Plant materials in half of each box were covered again with paleosol to simulate the conditions under which ground squirrels store plants. The other half was left uncovered to simulate decomposition on the soil surface (Fig. 3-3a). Each box was labeled with the name of its species and placed outside. To minimize disturbance, all boxes were covered with a screen (7-8 mm mesh) (Fig. 3-3b).

The wooden boxes and their contents were left outside at the University of Western Ontario greenhouses, London, Ontario, for 317 days (from October 21, 2013 to September 2, 2014). The plant tissues were sampled 4 times over the course of this experiment at days 1, 164, 253 and 317. The first sampling (October 21, 2013) was performed to record the initial C and N isotopic and elemental compositions of plants. The second sampling was performed following a hard winter (April 2, 2014) and two more samplings were performed over the summer (June 30 and September 2, 2014). At each sampling time, plant tissues were collected from different spots in each box: three from the covered portion and three from the uncovered portion. These samples were then washed with distilled water (DW), oven dried (at 90°C), ground and stored in sample glass vials for C and N isotopic and elemental analysis.

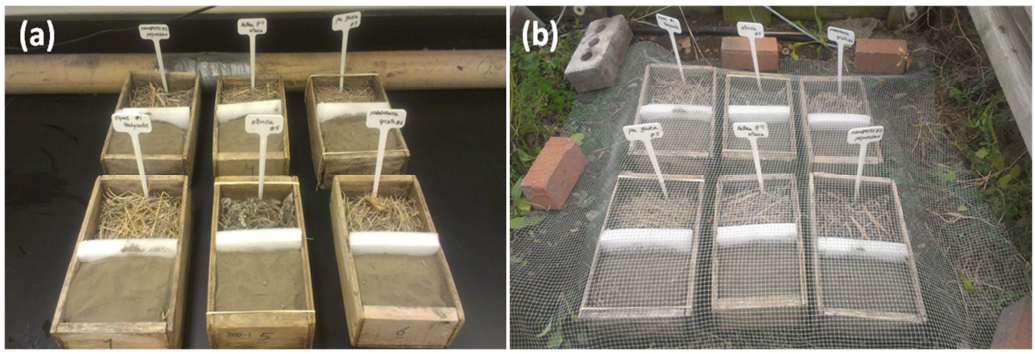


**Figure 3-3: Decomposition experiment (a) before and (b) after outdoor placement.**

### 3.3.2 Sample Collection

#### 3.3.2.1 Fossil Samples

A total of 24 fossil ground squirrel nests (Fig. 3-4) and 8 paleosol samples were collected in summer 2013 from two main sites: Quartz Creek (QC) and Independence Creek (IC) in the Klondike placer goldmine area of west-central Yukon Territory (Fig. 3-2). In addition, 12 fossil ground squirrel nests previously collected by G. Zazula from different locations in earlier years were obtained from Yukon Paleontology Collections of Department of Tourism and Culture. The locations and years of sampling for all nests are listed in Table 3-1. At all sites, placer gold mining provided a series of mining cuts (25-500 m length) that presented frozen "muck" (containing many plant and animal macrofossils) sitting on gold-bearing fluvial deposits (Fig. 3-5). Fossil ground squirrel nests and paleosols were collected from different depths along these mining cuts. Some sites (QC, EC and SC; Fig. 3-2) were associated with tephra layers (Dawson tephra, ca. 25,300 $^{14}$C a BP, Froese et al., 2006; and Sheep Creek tephra, ca. 80 ka, Westgate et al., 2008). Because the identification of the tephra layer was not certain at different sites, several samples from each site were selected for radiocarbon dating.

At QC, a dark soil layer interbedded between lighter colored layers was observed and three soil samples (Q-2, Q-4, and Q-3) obtained across this sequence with the idea that it might record a glacial-interglacial cycle (Fig. 3-6). The other soil samples were taken randomly from different depths. Bulk soil samples were collected from freshly cleaned

sections that had been partially or completely thawed for a few days, except one sample from IC, which was still completely frozen when collected.

Fossil nests and paleosols were placed in plastic bags and woven poly bags, respectively. Fossil nests were kept frozen in coolers using Dry Ice Gel Packs. All samples were transported by air to the Laboratory for Stable Isotope Science (LSIS), The University of Western Ontario (London, ON, Canada) to be prepared for botanical, elemental and isotopic analysis. In the laboratory, all samples were kept in a walk-in freezer (–25°C) until required for analysis. Most fossil nests were completely frozen at the time of sampling and so labeled (frozen or F). Samples or portions of samples, that had thawed were labeled differently as thawed (T). In some cases, some parts of a single nest were frozen (buried in sediments), but thawed at its exposure to the mining cuts. Both frozen and thawed portions were analyzed separately. If no significant isotopic and/or elemental differences were observed between the frozen and thawed parts of a nest, an average value is reported.

All fossil nests were freeze-dried prior to any analyses. They were then searched one by one for any distinctive macrofossils (seeds, leaves, stems, bones, insects, hair). Plant macrofossils were then examined using a binocular microscope, identified to the closest possible taxonomic resolution by comparison with previously identified materials (Zazula et al., 2003, 2005, 2007), and photographed. Bone samples were collected for identification, collagen extraction, and C and N isotopic and elemental analyses. Plant (herbaceous leaf, stem and wood) and/or bone (where available) samples from 10 nests plus two pieces of wood from the QC and IC sites were isolated and submitted to the NSF-Accelerator Mass Spectrometry facility at the University of Arizona, Tuscon Arizon, USA, for radiocarbon dating. Clean ice was also sampled from some nests for O and H isotopic analysis.

**Figure 3-4: Arctic ground squirrel nest found in perennially frozen paleosol in Quartz Creek (photographic credit: Fred Longstaffe).**

**Figure 3-5: Mining cuts at Quartz Creek (photographic credit: Fred Longstaffe).**

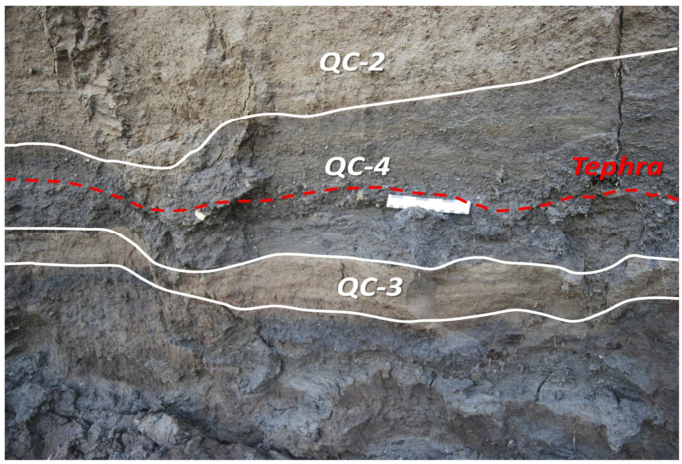


**Figure 3-6: A sequence of paleosol layers at Quartz Creek (photographic credit: Fred Longstaffe).**

#### 3.3.2.2 Modern Samples

The collection, preparation, and isotopic and elemental analyses of modern plants from the eastern shoreline of Kluane Lake and the Whitehorse area have been described in Chapter 2. Modern ground squirrel bones were also collected from these areas: (i) 3 individual bones from eastern Kluane Lake, which were collected in summer 2013, and (ii) 11 modern ground squirrel bones from carcasses provided by the Animal Health Laboratory in Environment Yukon, which were collected from Whitehorse international Eric Neilson airport in spring 2014. In the latter case, samples were taken from the right leg bones (femur, tibia, fibula). All soft tissues were removed using a knife and then a carbide burr Dremel tool attachment. There was no exposure of the bone to water, heat, or any protein de-naturing enzyme commonly used in defleshing.

### 3.3.3 Sample Preparation

#### 3.3.3.1 Fossil Plants

To prepare fossil plants for elemental and isotopic analyses, visibly well-preserved plant materials were sampled from each nest and soaked in DW 3 times, each time for 1-2 minutes to disperse attached sediment. The samples were then washed on top of a screen using DW and oven-dried at 90°C overnight. The oven-dried samples were ground using a Wig-L-Bug® (Crescent) and stored in small sealed glass vials while awaiting analysis. Then, in preparation for $\delta^{13}C$ and $\delta^{15}N$ measurements, 0.4 mg and 1-5 mg from each sample was loaded into tin capsules, respectively.

#### 3.3.3.2 Bone (Collagen Extraction)

Collagen extraction was done using a modified Longin (1971) method. Briefly, bone fragments were cleaned up with a wire brush and/or a brief treatment (3-4 minutes) in an ultrasonic bath to remove any dirt. For modern bones, the soft tissues were removed using a knife and then a carbide burr Dremel tool attachment. Cortical bones were mainly targeted, so in any cases where trabecular bones presented, these materials were removed using a carbide burr Dremel tool attachment. Cortical bone samples then were crushed to coarse powder (0.85mm and 0.18mm) and weighed out (at least 0.1 gr) into a glassy

microcentrifuge vial. All chemical treatments were performed at room temperature (ca. 20°C) unless otherwise noted. Samples then were treated with 1:2 chloroform:methanol solution for 15 minutes under fume hood to extract lipids (3 times). After drying, samples were soaked in 0.25 M HCl for 24 h and then demineralized in 0.5 M HCl, with frequent acid changes. When the samples were fully demineralised (soft to gummy), they were neutralized with DW (5-6 rinses), and then soaked in 0.1M NaOH for successive 20 min to remove humic substances. After several times (at least 6 times) rinsing samples with DW to neutrality, 8 ml 0.25 M HCl was added to samples to drop pH. Then, the acid was sucked off and samples left with 3 ml DW in the oven at 90°C for at least 16 h to solubilise collagen in acidic water. After 16 hours, the top liquid containing collagen was deposited into 4 ml glass vials and oven-dried at 90°C (at least for 24-36 h). Once the extracted collagen was completely dry, it was removed from the oven and the collagen yield determined as follows:

Equation 3.4

$$\% \text{ Yield} = [(\text{vial} + \text{collagen weight} - \text{vial weight}) / \text{Initial dry weight of bone}] \times 100$$

The collagen was then removed from glass vial using a dental pick, gently ground in a small mortar to homogenize the sample, and then ~0.4 mg of each sample was loaded into tin capsules in preparation for N and C isotope and elemental analysis.

#### 3.3.3.3 Bone (Bioapatite Structural Carbonate)

Prior to isotopic analysis of bone bioapatite structural carbonate, each bone sample was assessed for *post-mortem* recrystallization, deposition of secondary carbonate, and bioapatite carbonate to phosphate ratio (C/P) using Fourier Transform Infrared Spectroscopy (FTIR). Such testing is performed to help verify that isotopic compositions acquired during life were preserved after death (Shemesh, 1990; Munro et al., 2007; Wright and Schwarcz, 1996). Approximately 2 mg of finely powdered bone (grain size 45-65 $\mu$m) was mixed with 200 mg of KBr and compressed with a hydraulic press at 10 tons for 10 minutes to create a 12 mm pellet. Absorbance spectra were obtained using a Bruker Vector 22 FTIR Spectrometer, scanning 16 times from 400 to 4000 $cm^{-1}$, with a

resolution of 4 $cm^{-1}$. Spectra were compared to those obtained in previous studies for bone (Boyar et al., 2004; Pucéat et al., 2004) to determine whether any unusual phases were present. Crystallinity Indices (CI) and C/P were calculated following Weiner and Bar-Yosef (1990) and Pucéat et al. (2004), respectively. Precision was ± 0.11 and ± 0.08 for CI indices and C/P, respectively. Secondary carbonate was not detected in any sample, and therefore no treatment for carbonate (or OM) removal was performed prior to isotopic analysis.

Bone fractions that passed through a 0.18 mm sieve were weighed (0.5-1 mg) and then reacted with ortho-phosphoric acid ($H_3PO_4$) under vacuum at 90°C for 25 minutes using a Micro-mass MultiPrep automated sampling device. The $CO_2$ released from the bioapatite structural carbonate was then automatically transferred from the MultiPrep device to a coupled VG Optima IRMS in duel-inlet mode, where its carbon and oxygen isotopic compositions were determined.

#### 3.3.3.4 Water

Clean ice was sampled from fossil nests by wrapping them in aluminum foil and removing ice fragments using a clean screwdriver and hammer. The fragments were collected in small zip lock bags, wrapped and sealed using parafilm, and allowed to melt at room temperature. The water was then transferred to small glass vials, which were filled completely, sealed using parafilm and refrigerated prior to analysis of $\delta^{18}O$ and $\delta^{2}H$. Two modern water samples, one from Schwatka Lake, a dammed portion of the Yukon River in Whitehorse, and the other from Christmas Creek, which drains into Kluane Lake, were also sampled in September, 2012, using well-rinsed, polypropylene bottles that were filled completely, sealed with parafilm, kept cool, and transported within a few days to the LSIS laboratory for analysis of $\delta^{18}O$ and $\delta^{2}H$.

#### 3.3.3.5 Paleosols

The preparation and analysis of paleosols for physical and chemical characterization (amount of mineral fractions, OM, pH and mineralogy) followed methods described in Chapter 2 for modern soils. Carbonate removal from these samples was performed using

acid fumigation. About 3-15 mg of acid-fumigated samples then was weighed out into tin capsules in preparation for measurement of OC content and $\delta^{13}C$. For measurement of TN and TC contents, and $\delta^{15}N$, about 5-15 mg un-acidified samples were loaded into tin capsules because acid-treatment can affect these measures (Harris et al., 2001).

### 3.3.4 Elemental Analysis

The OC and TN contents (dry wt. %) of fossil plant samples were determined following the same protocols described in Chapter 2 for modern plants. The OC and TN abundances were calibrated using USGS40 (glutamic acid, accepted value: C = 40.78 wt. %, N = 9.50 wt. %) and USGS41 (glutamic acid, accepted value: C = 40.78 wt. %, N = 9.50 wt. %). The accuracy and precision were determined using the internal laboratory standard keratin for C and N (accepted values: 48.22 ± 1.07 wt. % (n = 28) for C and 14.85 ± 0.43 wt. % (n = 261) for N) and NIST 1547 (Peach Leaves) for N (certified value: 2.94 wt. %). For some analytical sessions with accuracy outside of the acceptable range (within ± 1 % for C and within ± 0.5 % for N), the results were recalibrated using data from separate analyses performed using a Fisons 1108 EA (Appendix B). Any samples for which both precision and accuracy were outside of acceptable limits (> ± 1 wt. % for C and > ± 0.5 wt. % for N) were reanalyzed using the Fisons 1108 EA. Samples analyzed using the Fisons 1108 EA were calibrated to the laboratory acetanilide standard ($C_6H_5NH(COCH_3)$: accepted values: C = 71.09 wt. %, N = 10.36 wt. %). The accepted elemental data and other information about these reference materials are provided in Appendix A. All elemental analysis analytical sessions were calibrated to accepted values of standards to two decimal places, with the results being reported to one decimal place considering the SD of replicate measurements.

Following reanalysis of problematic samples, the average C and N contents for keratin standard was 47.74 ± 0.97 wt. % (n = 58) and 14.26 ± 0.44 % (n = 113), respectively, which compare well to their expected values of 48.22 ± 1.07 wt. % and 14.85 ± 0.43 wt. %. Reproducibility of duplicate samples for C was ± 0.43 wt. % (n = 23). The average N content for NIST 1547 was 2.72 ± 0.07 wt. % (n = 50), which compares well with its

accepted value of 2.94 wt. %. Reproducibility of duplicate samples for N was ± 0.02 wt. % (n = 30).

### 3.3.5 Stable Isotope Analyses

All stable isotopic results are presented using $\delta$-notation (Coplen, 2011):

Equation 3.5 $\delta^{13}C, \delta^{15}N, \delta^{18}O$ or $\delta^{2}H$ (‰) = $[(R_{Sa}/R_{Std}) - 1]$

where $R_{Sa}$ and $R_{Std}$ denote $^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{18}O/^{16}O$ or $^{2}H/^{1}H$ in the sample and standard (VPDB for carbon, AIR for nitrogen, VSMOW for oxygen and hydrogen), respectively. Analytical accuracy and precision for all standards associated with data presented in this chapter are listed in Appendix G. All sample results are reported to one decimal place, consistent with the SD of replicate measurements, except for $\delta^{2}H$ which are reported to the nearest 1 ‰.

The C and N isotopic compositions of bone collagen, plants, and soil OC (after IC removal) and soil TN were measured following the protocol described in Chapter 2. The $\delta^{13}C$ and $\delta^{15}N$ of all samples were calibrated to VPDB (C) and AIR (N) using USGS40 and USGS41 (see Chapter 2, section 2.2.3). Accuracy and precision were monitored using the laboratory keratin and IAEA-CH-6 (sucrose). The average $\delta^{13}C$ obtained for keratin was –24.04 ± 0.07 ‰ (n = 75), which compares well with its accepted value of –24.05 ± 0.15 ‰. The average $\delta^{13}C$ obtained for IAEA-CH-6 was –10.50 ± 0.09 ‰ (n = 26), which compares well with its accepted value –10.45 ± 0.03 ‰ (Coplen et al., 2006). Reproducibility for duplicate samples was ± 0.15 ‰ for $\delta^{13}C$ (n = 26). The average $\delta^{15}N$ of keratin was +6.42 ± 0.12 ‰ (n = 113), which compares well with its accepted value of +6.36 ‰. Sample reproducibility was ± 0.08 ‰ for $\delta^{15}N$ (n = 32).

The $\delta^{13}C$ results for bone bioapatite structural carbonate were calibrated relative to VPDB using NBS-19 (accepted value: $\delta^{13}C$ = +1.95 ‰) and LSVEC (accepted value: $\delta^{13}C$ = –46.60 ± 0.2 ‰); the $\delta^{18}O$ values were calibrated relative to VSMOW using NBS-19 (accepted value: +28.60 ‰) and NBS-18 (accepted value: +7.20 ‰) (Coplen et al., 2006). Accuracy and precision were monitored using internal calcite standards WS-1 and

Suprapur. The average $\delta^{13}C$ obtained for WS-1 and Suprapur were +0.69 ± 0.11 ‰ (n = 3) and –35.78 ± 0.01 ‰ (n = 2), respectively, which compare well with their accepted values +0.8 ‰ and –35.55 ‰, respectively. The average $\delta^{18}O$ obtained for WS-1 and Suprapur were +26.19 ± 0.14 ‰ and +13.28 ± 0.08, respectively, which compares well with their accepted values +26.2 ‰ and +13.3 ‰, respectively. Reproducibility for duplicate samples was ± 0.22 ‰ for $\delta^{13}C$ (n = 2) and ± 0.19 ‰ for $\delta^{18}O$ (n = 2).

The $\delta^{18}O$ of water samples was measured using a modified version of the $CO_2$-equilibrium method originally described by Epstein and Mayeda (1953). One ml of water was added to a septum-sealed glass vial, which was then placed in a Thermo Scientific GasBench heating block. The atmosphere inside the vials was flushed and replaced with 0.3 % $CO_2$ in He. Samples and standards were equilibrated at 35°C for at least 3-4 days. The equilibrated $CO_2$ was then automatically injected from vials in GasBench device to a coupled Delta$^{plus}$ XL-CF-IRMS in duel-inlet mode, where its $\delta^{18}O$ were determined.

The $\delta^{2}H$ of water was determined using the $H_2$-equilibration method of Horita (1988). One ml of water was added to a septum-sealed glass vial, to which was also added a reusable platinum catalyst known as a “Hokko stick” (Shoko Co. Ltd, Japan). The vials containing the samples and standards were then placed in a Thermo Scientific GasBench heating block and equilibrated with 2 % $H_2$ in He at 35°C for approximately 2 hours. The equilibrated $H_2$ was then automatically transferred from the GasBench device to a coupled Delta$^{plus}$ XL-CF-IRMS in duel-inlet mode, where its $\delta^{2}H$ were determined.

The $\delta^{18}O$ and $\delta^{2}H$ values were calibrated relative to VSMOW using internal laboratory standards that had been previously calibrated to VSMOW and SLAP (Coplen, 1988): LSD ($\delta^{18}O$ = –22.57 ‰, $\delta^{2}H$ = –161.8 ‰) and HEAVEN ($\delta^{18}O$ = –0.27 ‰, $\delta^{2}H$ = +88.7 ‰). Accuracy and precision were monitored using internal water standards EDT and MID for water samples analysis. The average $\delta^{18}O$ and $\delta^{2}H$ values obtained for EDT were –7.22 ± 0.10 ‰ (n = 2) and –55.0 ± 1.0 ‰ (n = 4), respectively, which compare well with their accepted values –7.27 and –56.0 ‰, respectively. The average $\delta^{18}O$ and $\delta^{2}H$ obtained for MID were –13.03 ± 0.05 ‰ (n = 2) and –107.37 ± 1.93 (n = 3),

respectively, which compare well with their accepted values –13.08 ‰ and –108.1 ‰, respectively.

### 3.3.6 Microscopic Analysis

Fossil plants and decomposed modern plants sampled at Day 317 were cleaned using a soft toothbrush, washed using DW to remove contaminants, and then freeze-dried. The samples were then examined using a Leica S8APO-MDG41 dissecting microscope in order to identify their botanical taxonomy and, for the decomposition experiment, to track any changes in texture and color. Portions of each sample were also mounted on an aluminum stub and coated with gold palladium alloy in preparation for Scanning Electron Microscopy (SEM). SEM photomicrographs (50-1000× magnification) were obtained using a Hitachi S3400N electron microscope operated at 25.0 kV.

### 3.3.7 Statistical Analysis

Changes in the $\delta^{13}C$ and $\delta^{15}N$ of plant detritus over the 317 days of the decomposition experiment were tested using repeated measures ANOVA (general linear model) and applying the Greenhouse-Geisser correction. When the effect of time on $\delta^{13}C$ and $\delta^{15}N$ was shown to be significant, the Bonferroni *post hoc* test was used to perform Pairwise Comparisons to determine at what point the significant difference in $\delta^{13}C$ and $\delta^{15}N$ occurred. Possible correlations between (i) soil OC and OM contents, (ii) decomposed plant $\delta^{15}N$ and C/N, (iii) decomposed plant $\delta^{15}N$ and C content, and (iv) decomposed plant C/N and C content were assessed using Pearson's rank correlation coefficient (R). All statistical analyses were performed in SPSS 20.

## 3.4 Results

### 3.4.1 Paleosols

General information for each sampling site is presented in Table 3-1. Eight paleosols (7 from QC and 1 from IC) were sampled at exposures created by recent placer mining (Fig. 3-5). These paleosols were presented on north-facing slopes with the upper limit of permafrost located within a few meters of the land surface. All soil samples were

analyzed for basic physical and chemical properties plus OC and TN isotopic compositions. The results of these analyses are presented in Tables 3-2, 3-3 and 3-4.

All samples are dominated by silt (> 57 wt. %, avg. 69.5 ± 6.7 wt. %). Mean clay and sand contents are 17.2 wt. % and 13.4 wt. %, respectively. Based on this analysis, all paleosol samples are classified as Silty Loam. The OM content ranges from 5.0 to 20.4 wt. %. For all samples, there is a strong correlation between OC and OM contents (loss on ignition ($LOI_{550}$)) (R = 0.998, *p*-value = 0.000).

The paleosols have a wide range in OC content (1.8-9.5 wt. %). The range in pH, by comparison, is very small (7.1-7.9) and indicates neutral to alkaline conditions (avg. 7.6 ± 0.3). The bulk paleosol mineralogy consists mainly of quartz, feldspar and calcite with amphibole and mica present in a few samples (Table 3-3). The paleosol $\delta^{15}N$ ranges from +1.3 ‰ in QC-4 to +4.8 ‰ in IC-9. The variation in $\delta^{13}C$ is smaller (–26.1 to –25.4 ‰).

**Table 3-1: General data for sampling sites.**

| Site ID | Site Location | Latitude | Longitude | Sampling Year | Nests sampled | Soil sampled | $^{14}$C date |
|---|---|---|---|---|---|---|---|
| QC | Quartz Creek | 63.7513 | -139.1252 | 2012/2013 | 11 | 7 | > 49,900 |
| IC | Independence Creek | 63.9831 | -139.0212 | 2013 | 16 | 1 | > 41,200 to 22,520 |
| LB | Little Blanche Creek | 63.8312 | -139.0872 | 2009 | 1 | - | > 40,300 |
| EC | Eureka Creek | 63.6300 | -138.8251 | 2011 | 2 | - | 26,530 |
| GC | Glacier Creek[a] | 64.0368 | -140.8195 | 2013 | 1 | - | 16,580 |
| SC | Sulphur Creek[b] | 63.6480 | -138.6710 | 2011 | 5 | - | 21,180 |

**[a] Sixty mile River area.**

**[b] Dan Klipper/Rod Smith Placer mine.**

***All placer mines are located in Yukon Territory, Canada.**

**$^{14}$C age for each site has been estimated based on the oldest and youngest dates obtained for each site.**

**Table 3-2: Paleosol characteristics.**

| Sample ID | pH | Texture | Sand | Silt | Clay | OM | OC | TC | TN | Atomic OC/TN |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | wt. % | | | | | | | |
| QC-2 | 7.6 | SiL | 1.6 | 78.0 | 20.4 | 5.8 | 2.0 | 2.4 | 0.2 | 12.1 |
| QC-3 | 7.3 | SiL | 29.6 | 57.6 | 12.8 | 12.2 | 5.0 | 5.2 | 0.4 | 13.9 |
| QC-4 | 7.1 | SiL | 11.6 | 67.6 | 20.8 | 20.4 | 9.5 | 9.0 | 0.6 | 17.6 |
| QC-5 | 7.9 | SiL | 13.6 | 71.6 | 14.8 | 5.4 | 1.8 | 2.3 | 0.2 | 10.8 |
| QC-6 | 7.8 | SiL | 7.6 | 75.6 | 16.8 | 5.4 | 1.9 | 2.3 | 0.2 | 11.4 |
| QC-7 | 7.8 | SiL | 9.6 | 73.6 | 16.8 | 6.6 | 2.4 | 2.7 | 0.2 | 11.7 |
| QC-8 | 7.8 | SiL | 15.6 | 67.6 | 16.8 | 6.0 | 1.9 | 2.3 | 0.2 | 11.7 |
| IC-9 | 7.5 | SiL | 17.6 | 64.0 | 18.4 | 5.0 | 1.9 | 2.2 | 0.2 | 12.5 |

**OM: Organic Matter; OC: Organic Carbon; TC: Total Carbon; TN: Total Nitrogen; SiL: Silty Loam**

**Table 3-3: Paleosol mineralogy.**

| Sample ID | Quartz | Ca, Na-Feldspar | Calcite | Chlorite/Vermiculite | Amphibole | Mica | Dolomite |
|---|---|---|---|---|---|---|---|
| | wt. % | | | | | | |
| QC-2 | 88 | 8 | < 5 | < 5 | - | - | - |
| QC-3 | 75 | 20 | - | < 5 | - | - | - |
| QC-4 | 83 | 11 | - | 6 | - | - | - |
| QC-5 | 79 | 7 | < 5 | 6 | - | - | 7 |
| QC-6 | 76 | 13 | < 5 | < 5 | < 5 | < 5 | - |
| QC-7 | 68 | 17 | < 5 | 7 | < 5 | < 5 | - |
| QC-8 | 79 | 9 | 5.0 | < 5 | < 5 | < 5 | - |
| IC-9 | 86 | 11 | - | - | - | < 5 | - |

**Table 3-4: Isotopic composition of soil TN and OC.**

| Sample ID | $\delta^{15}$N (‰, AIR) | $\delta^{13}$C (‰, VPDB) |
|---|---|---|
| **QC-2** | +4.6 | −25.8 |
| **QC-3** | +3.4 | −25.7 |
| **QC-4** | +1.3 | −26.1 |
| **QC-5** | +4.8 | −25.6 |
| **QC-6** | +4.7 | −25.4 |
| **QC-7** | +4.3 | −25.4 |
| **QC-8** | +4.5 | −25.4 |
| **IC-9** | +4.8 | −25.4 |

### 3.4.2 Fossil Plants

#### 3.4.2.1 Botanical Compositions

Typical plant macrofossils recovered from the study are shown in Figures 3-7 and 3-8, and are consistent with previously reported results for late Pleistocene plant macrofossils from the region (Wooller et al., 2011; Zazula et al., 2006b, 2007). The samples are dominated by the floret from grasses (*Alopecurus* sp., *Deschampsia caespitose* and *Carex* spp.) and the dried fruits of forbs (*Taraxacum* sp., *Draba* sp., *Ranunculus* sp., *Lepidium densiflorum* and *Plantago* cf. *canescens*) and suggest that late Pleistocene Beringia was a grass- and forb-dominated ecosystem.


**Figure 3-7: Typical plant macrofossils recovered from fossil nests. (a) *Carex* floret; (b) *Campylium stellatum* stem with leaves; (c) *Conioselinum cnidiifolium* carpel; (d) *Asteracea* achenese, pappus; (e) *Lepidium densiflorum*; (f) *Phlox hoodii* capsule; (g) *Taraxacum ceratophorum* achene; (h) *Pedicularis* sp. achene; (i, j) *Silene cf. taymirensis* capsule with seeds inside; (k) *Polemonium* capsule; (l, n) *Ranunculuc pensylvanicus-macounii* achene; (m) *Draba* spp. capsule; (o) *Plantago* cf. *canescens* capsule.**

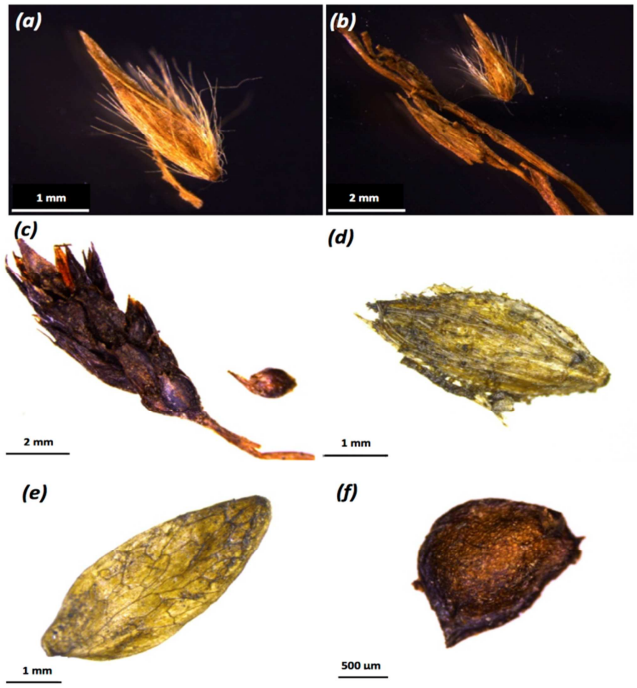


**Figure 3-8: Typical plant macrofossils recovered from fossil ground squirrel nests. (a) *Deschampsia caespitose* floret; (b) *Deschampsia caespitose* floret with stem; (c) *Carex albo niagra* floret and seed; (d) *Alopecurus* sp. Floret; (e) *Draba* sp. Silique; (f) *Ranunculus eschscholtzii-sulphureus* achene.**

### 3.4.2.2 Isotopic and Elemental Compositions of Fossil Plants

The isotopic and elemental data for bulk samples from each fossil squirrel nest are presented in Table 3-5. The $\delta^{13}C$ of samples from the QC site ranges from –26.7 to –24.1 ‰ (avg. –25.7 ‰), and from the IC site, from –27.6 to –25.2 ‰ (avg. –26.3 ‰). The range of $\delta^{13}C$ is similar for the other sites. The samples show much larger variation in $\delta^{15}N$, ranging from +1.8 to +9.3 ‰ (avg. +4.1 ‰) at QC, +1.2 to +10.6 ‰ (avg. +5.8 ‰) at IC, and from +1.0 to +13.6 ‰ collectively over the other sites. Fossil plants from nests with both frozen and thawed portions show some variations in C and N isotopic compositions which could be related to microbially mediated decay and/or heterogeneity of nest materials. The N and C contents of all samples range from 1.0 to 2.8 wt. % and 21.2 to 39.5 wt. %, respectively. The average atomic C/N of all samples is 24.2 ± 6.2.

### 3.4.3 Radiocarbon Dates

Table 3-5 lists the radiocarbon dates obtained for 10 fossil nests, along with the estimated age for other nests based upon their relative position to dated nests. The estimated dates for each placer goldmine visited are listed in Table 3-1 based on the youngest and oldest dates obtained for each site. Dates obtained for two specimens from QC are beyond the limits of the radiocarbon dating technique (> 49,900 $^{14}$C a BP). Dates for specimens from IC fall within a wide range (> 41,200 to 22,520 $^{14}$C a BP). Only one nest was dated from the rest of sites examined (LB, EC, GC and SC). No attempt was made to calibrate the $^{14}$C dates obtained in this study.

### 3.4.4 Water Isotopic Composition

The $\delta^{18}$O and $\delta^{2}$H for ice collected from fossil squirrel nests ranges from –30.7 to –26.6 ‰ (avg. –29.1 ± 1.6 ‰) and –242 to –219 ‰ (avg. –231 ± 9 ‰), respectively (Table 3-6). The $\delta^{18}$O and $\delta^{2}$H of modern water from Schwatka Lake and Christmas Creek are –19.4 and –149 ‰, and –22.5 and –173 ‰, respectively.

### 3.4.5 Modern Plants

All C and N isotopic data for modern plants discussed in this chapter were collected from the eastern shoreline of Kluane Lake and the Whitehorse area, as discussed in Chapter 2. The average $\delta^{13}$C and $\delta^{15}$N of all plant parts sampled in each study area has been utilized to define the modern isotopic baselines. Average values at Kluane Lake are: $\delta^{13}$C = –27.1 ± 1.2 ‰ (n = 207), and $\delta^{15}$N = –0.1 ± 2.2 ‰ (n = 207) (Appendix D). Average values for the Whitehorse area are: $\delta^{13}$C = –27.8 ‰ ± 1.2 ‰ (n = 115), and $\delta^{15}$N = +0.2 ‰ ± 2.2 ‰ (n = 115) (Appendix D). Isotopic data for plant samples from sites S12-6, S13-11, S13-13, S13-14 and S13-15 have not been included in these averages, as they are located far from the sites of bone sampling.

**Table 3-5: C and N isotopic and elemental compositions of bulk plants recovered from fossil nests.**

| Nest ID[1] | Sampling Year | $\delta^{13}$C (‰) | $\delta^{15}$N (‰) | C (wt. %) | N (wt. %) | Atomic C/N | $^{14}$C date |
|---|---|---|---|---|---|---|---|
| **QC-1-T** | **2013** | −26.6 | +4.6 | 29.5 | 1.7 | 20.8 | > 49,900 |
| **QC-2-F** | **2013** | −24.1 | +2.1 | 31.4 | 1.4 | 25.8 | > 49,900 |
| **QC-3-F** | **2013** | −26.2 | +1.1 | 28.1 | 1.6 | 20.4 | > 49,900 |
| **QC-3-T** | **2013** | −26.1 | +2.4 | 33.5 | 1.8 | 21.8 | > 49,900 |
| **QC-4-T** | **2013** | −26.1 | +1.9 | 31.4 | 1.6 | 22.4 | **> 49,900** |
| **QC-5-F** | **2013** | −26.1 | +1.8 | 32.2 | 2.0 | 19.0 | > 49,900 |
| **QC-5-T** | **2013** | −27.7 | +2.1 | 32.1 | 2.0 | 18.5 | > 49,900 |
| **QC-7-F** | **2013** | −26.2 | +2.6 | 38.7 | 1.1 | 39.6 | > 49,900 |
| **QC-8-F** | **2013** | −25.6 | **+7.5** | 36.0 | **1.8** | 22.9 | > 49,900 |
| **QC-9-F** | **2013** | −25.6 | +9.3 | 32.3 | 2.8 | 13.4 | > 49,900 |
| **QC-GZ-2-3** | **2012** | −26.1 | +6.6 | 39.5 | 1.3 | 36.2 | > 49,900 |
| **QC-GZ-5-9** | **2012** | −26.4 | +3.9 | 37.9 | 1.4 | 32.3 | > 49,900 |
| **QC-GZ-1** | **2012** | −25.7 | +3.3 | 29.8 | 1.3 | 26.9 | > 49,900 |
| **IC-1-F** | **2013** | −26.7 | +10.3 | 31.5 | 1.9 | 19.8 | **24,800** |
| **IC-1-T** | **2013** | −25.6 | **+9.0** | 39.2 | **2.2** | 20.4 | **24,800** |
| **IC-2-F** | **2013** | −26.6 | +6.2 | 30.8 | 1.6 | 22.2 | < 24,800 |
| **IC-3-F** | **2013** | −26.4 | +10.6 | 36.2 | 2.1 | 20.2 | **22,940** |
| **IC-4-F** | **2013** | −26.9 | +1.2 | 28.6 | 1.6 | 21.3 | > 41K - 22k |
| **IC-5-F** | **2013** | −26.5 | +9.2 | 35.8 | 1.8 | 23.7 | > 41K - 22k |
| **IC-6-F** | **2013** | −27.6 | +10.0 | 30.2 | 1.4 | 26.1 | > 41K - 22k |

**Table 3-5. Cont'd.**

| Nest ID[1] | Sampling Year | $\delta^{13}$C (‰) | $\delta^{15}$N (‰) | C (wt. %) | N (wt. %) | Atomic C/N | $^{14}$C date |
|---|---|---|---|---|---|---|---|
| **IC-7-F** | **2013** | –25.7 | +4.6 | 38.1 | 1.4 | 31.1 | > 41K - 22k |
| **IC-7-T** | **2013** | –25.3 | +4.3 | 25.4 | 1.6 | 18.9 | > 41K - 22k |
| **IC-8-F** | **2013** | –25.9 | +6.3 | **36.0** | **1.6** | 25.6 | > 41K - 22k |
| **IC-8-T** | **2013** | –26.8 | +8.5 | **33.5** | **1.7** | 22.7 | > 41K - 22k |
| **IC-9-F** | **2013** | –27.0 | +2.4 | 34.9 | 1.8 | 22.6 | > 41K - 22k |
| **IC-9-T** | **2013** | –26.3 | +1.9 | 36.1 | 1.7 | 24.2 | > 41K - 22k |
| **IC-10-F** | **2013** | **–26.0** | **+2.7** | 33.6 | **1.7** | 23.8 | > 41K - 22k |
| **IC-11-F** | **2013** | –26.4 | +4.2 | 32.8 | 1.4 | 28.3 | > 41K - 22k |
| **IC-12-F** | **2013** | –26.3 | +5.2 | 34.0 | 1.7 | 23.7 | > 41K - 22k |
| **IC-13-T** | **2013** | –25.2 | +3.4 | 36.5 | 1.8 | 24.1 | > 41K - 22k |
| **IC-14-F** | **2013** | –26.2 | +4.4 | 36.0 | 1.7 | 24.2 | **> 41,200** |
| **IC-14-T** | **2013** | –26.1 | +3.6 | 30.8 | 1.6 | 23.1 | **> 41,200** |
| **IC-15-T** | **2013** | –25.9 | +2.1 | **25.8** | **1.5** | 19.8 | > 41K - 22k |
| **IC-19-T** | **2013** | **–26.2** | +6.3 | 35.8 | 1.7 | 24.6 | **22,520** |
| **LB-GZ-1** | **2009** | –26.3 | +7.1 | 31.4 | 2.0 | 18.5 | **> 40,300** |
| **GC-GZ-3** | **2013** | –25.7 | +7.4 | 21.2 | 2.1 | 11.8 | **16,580** |
| **EC-GZ-6** | **2011** | **–25.6** | +7.5 | 36.6 | 1.7 | 24.8 | > 26,000 |
| **EC-GZ-2** | **2011** | –26.8 | +4.8 | 29.0 | 1.9 | 18.1 | **26,530** |
| **SC-GZ-8** | **2011** | –24.9 | +5.8 | 29.3 | 1.7 | 20.7 | **~** 21,180 |
| **SC-GZ-7** | **2011** | –26.5 | +1.0 | 38.7 | 1.0 | 44.3 | **~** 21,180 |

**Table 3-5. Cont'd.**

| Nest ID[1] | Sampling Year | $\delta^{13}$C (‰) | $\delta^{15}$N (‰) | C (wt. %) | N (wt. %) | Atomic C/N | $^{14}$C date |
|---|---|---|---|---|---|---|---|
| **SC-GZ-4** | **2011** | −26.2 | +5.2 | 30.1 | 1.3 | 27.8 | > 26,000 |
| **SC-GZ-2** | **2011** | −24.7 | +8.5 | **38.9** | **1.7** | 27.3 | **~** 21,180 |
| **SC-GZ-10** | **2011** | −26.0 | +13.6 | 33.4 | 1.5 | 26.3 | **21,180** |

**[1]: Nest IDs are based on site locations summarized in Table 3-1.**
**F: Frozen at the time of sampling; T: Thawed at the time of sampling.**
**Isotopic and elemental results in boldface font are average of duplicates.**
**Dates in boldface font are measured $^{14}$C AMS dates; other dates are estimated based on relative location (above/below) to dated nests.**

**Table 3-6: $\delta^{18}O$ and $\delta^{2}H$ of water.**

| Nest ID | $\delta^{18}O$ | $\delta^{2}H$ | $^{14}C$ age |
|---|---|---|---|
| | (‰, VSMOW) | | |
| **Ancient (Klondike goldmines)** | | | |
| **QC-4** | −30.7 | −242 | > 49,900 |
| **QC-5** | **−30.2** | **−235** | > 49,900 |
| **IC-1** | −26.6 | −219 | 24,800 |
| **IC-4** | −30.0 | −236 | - |
| **IC-6** | −30.6 | −240 | - |
| **IC-9** | **−27.9** | −222 | 26,930 |
| **IC-10** | **−27.0** | **−220** | **-** |
| **IC-12** | **−29.5** | **−235** | - |
| **IC-14** | **−29.7** | −233 | > 41,200 |
| **Modern (Yukon Territory)** | | | |
| **Schwatka Lake[1]** | −19.4 | −149 | - |
| **Christmas Creek[2]** | −22.5 | −173 | - |

**[1]Latitude: 60.6919°, longitude: –135.0287°**
**[2]Latitude: 61.0591°, longitude: –138.3554°**
**Boldface font denotes averages for duplicates.**

### 3.4.6 Fossil and Modern Bone Isotopic Compositions

Bone samples recovered from the fossil ground squirrel nests varied in size and morphology. They can be categorized into two main groups of Arctic rodents: Arctic ground squirrels (IC-3, IC-19, IC-9-2) and lemmings (GZ-1, GZ-3-1, IC-9-1, QC-4) (Fig. 3-9). GZ-3-2 most probably belongs to a megaherbivore, but the species is unknown. Two separate bone samples were taken for collagen extraction from each of nests GZ-3 and IC-9, given the large observed differences in bone size and morphology (Fig. 3-9).

The C and N isotopic and elemental compositions of fossil and modern bone collagen are presented in Table 3-7. The C and N contents, atomic C/N and extraction yields of the bone collagen samples were used to evaluate collagen preservation. All extracts have N >

10 wt. %, atomic C/N from 2.9 to 3.6 and collagen yields > 2 %, which fit the criteria for well-preserved samples (Ambrose, 1990; DeNiro, 1985). The $\delta^{13}C_{Col}$ and $\delta^{15}N_{Col}$ range from –21.9 to –19.5 ‰ (avg. –21.1 ‰), and +3.9 to +5.6 ‰ (avg. +4.7 ‰), respectively, and show no significant variation with bone size or morphology. The $\delta^{13}C_{Col}$ and $\delta^{15}N_{Col}$ of the modern bones are lower, ranging from –24.2 to –21.2 ‰ (avg. –23.2 ‰), and +1.1 to +3.2 ‰ (avg. +2.1 ‰), respectively.


**Figure 3-9: Fossil bones recovered from fossil nests.**

The mean FTIR-CI value of modern and fossil bones are 2.5 ± 0.1 (n = 14) and 2.6 ± 0.2 (n = 8), respectively (Table 3-8), within the previously reported range for fresh (2.5-3.25) and archeological bone (2.6-4.5) (Garvie-Lok et al., 2004; Munro et al., 2007; Wright and Schwarcz, 1996). All CI values are < 3.8 (bones with CI > 3.8 are considered altered; Shemesh, 1990). The mean C/P of both modern and fossil bones, which is an estimate of structural carbonate preservation, is 0.7 ± 0.1 (Table 3-8), only slightly higher than commonly reported for well-preserved bone (~0.2-0.5) (Smith et al., 2007; Wright and Schwarcz, 1996). It is unlikely, however, that there is secondary carbonate contamination in these samples, as the modern bones were obtained directly from fresh carcasses without any pretreatment. Also, there is no discrete peak in the FTIR spectra at 710 and

1096 $cm^{-1}$, which would indicate secondary calcite or francolite deposition characteristic of *post-mortem* alteration (Shemesh, 1990; Wright and Schwarcz, 1996).

The $\delta^{13}C_{Sc}$ of fossil bone ranges from –14.5 to –12.0 ‰ (avg. –13.5 ‰), and the $\Delta^{13}C_{Sc\text{-}Col}$ varies from +6.9 to +8.7 ‰ (avg. +7.8 ‰) (Table 3-7). The $\delta^{13}C_{Sc}$ of the modern bone is significantly different between the two study areas, ranging from –9.6 to –8.8 ‰ (avg. –9.1 ‰) at Kluane Lake *vs*. –18.9 to –16.9 ‰ at Whitehorse (avg. –18.0 ‰). The $\Delta^{13}C_{Sc\text{-}Col}$ at Kluane Lake ranges 11.6 to 13.8 ‰ (avg. +12.7 ‰) *vs*. +5.0 to +6.2 ‰ (avg. +5.6 ‰) at Whitehorse.

The $\delta^{18}O_{Sc}$ of the fossil bone ranges from +10.9 to +14.6 ‰ (avg. +12.4 ‰), whereas it varies from +15.8 to +18.8 ‰ (avg. +17.3 ‰) for the modern samples at Kluane Lake, and from +22.3 to +27.4 ‰ (avg. +25.1 ‰) at Whitehorse (Table 3-7). Based on these data and Equations 3.2 and 3.3, the calculated average $\delta^{18}O_{dw}$ for the late Pleistocene fossil bone samples is –29.1 ± 2.8 ‰ (n = 8), and for modern bone –19.2 ± 3.0 ‰ (n = 3) at Kluane Lake and –3.7 ± 2.6 ‰ (n = 11) at Whitehorse (Table 3-9).

**Table 3-7: Fossil and modern bones collagen $\delta^{13}C$, $\delta^{15}N$, C and N contents (wt. %) and yield, and structural carbonate $\delta^{18}O$ and $\delta^{13}C$.**

| Bone ID | $\delta^{13}C_{Col}$ | $\delta^{13}C_{Sc}$ | $\Delta^{13}C_{Sc\text{-}Col}$ | $\delta^{15}N_{Col}$ | $\delta^{18}O_{SC}$ | C | N | C/N | Yield | $^{14}C$ date |
|---|---|---|---|---|---|---|---|---|---|---|
| | (‰, VPDB) | | | (‰, AIR) | (‰, VSMOW) | (wt. %) | | (atomic) | (%) | |
| **Fossil Bones** | | | | | | | | | | |
| **QC-4** | −21.4 | −13.1 | +8.3 | +4.8 | +10.9 | 37.6 | 13.2 | 3.3 | 8.5 | > 49,900 |
| **IC-3** | −21.4 | −14.5 | +6.9 | +5.1 | +11.0 | 32.8 | 11.5 | 3.3 | 4.5 | 22,940 |
| **IC-9-1** | −21.7 | **−14.3** | +7.4 | +4.0 | **+13.4** | 42.4 | 14.7 | 3.4 | 5.1 | 26,930 |
| **IC-9-2** | **−21.5** | **−14.0** | +7.6 | **+4.2** | **+13.4** | **39.6** | **13.8** | **3.4** | 6.5 | 26,930 |
| **IC-14** | −21.2 | −12.5 | +8.7 | +3.9 | +12.0 | 28.7 | 9.3* | 3.6 | 2.3 | 41,200 |
| **IC-19** | −21.2 | −13.2 | +8.0 | +4.6 | +14.6 | 43.8 | 15.9 | 3.2 | 15.3 | 22,520 |
| **LB-GZ-1** | −21.9 | −14.5 | +7.4 | +4.5 | +10.9 | 43.6 | 15.2 | 3.4 | 8.6 | > 40,300 |
| **GC-GZ-3-1** | **−19.9** | −12.0 | +7.9 | **+5.6** | **+12.7** | **42.4** | **15.0** | 3.3 | **9.1** | 16,580 |
| **GC-GZ-3-2** | **−19.5** | - | - | **+5.3** | - | **32.5** | **11.4** | 3.3 | **4.4** | 16,580 |
| **Modern Bones** | | | | | | | | | | **Sampling Site** |
| **M-1-female** | −23.9 | −18.3 | +5.4 | +1.3 | +24.1 | 48.3 | 16.8 | 3.4 | 15.4 | **WH** |
| **M-2-male** | −24.2 | **−18.7** | +5.6 | +2.8 | **+25.0** | 41.7 | 14.7 | 3.3 | 16.5 | **WH** |
| **M-3-male** | −24.0 | −17.7 | +6.1 | +2.8 | +26.2 | 47.9 | 16.7 | 3.4 | 15.7 | **WH** |
| **M-5-male** | −23.9 | −17.6 | +6.2 | +2.1 | +27.4 | 41.0 | 14.6 | 3.3 | 17.2 | **WH** |
| **M-6-male** | −22.7 | −16.9 | +5.9 | +2.3 | +25.4 | 42.2 | 14.7 | 3.4 | 16.4 | **WH** |
| **M-7-male** | −23.7 | −17.6 | +6.0 | +1.1 | +25.1 | 46.4 | 15.9 | 3.4 | 14.6 | **WH** |
| **M-8-female** | −23.8 | −18.3 | +5.4 | +1.9 | +24.0 | 47.5 | 16.5 | 3.4 | 15.3 | **WH** |

**Table 3.7 Cont'd.**

| Bone ID | $\delta^{13}C_{Col}$ | $\delta^{13}C_{Sc}$ | $\Delta^{13}C_{Sc\text{-}Col}$ | $\delta^{15}N_{Col}$ | $\delta^{18}O_{SC}$ | C | N | C/N | Yield | Sampling |
|---|---|---|---|---|---|---|---|---|---|---|
| | (‰, VPDB) | | | (‰, AIR) | (‰, VSMOW) | (wt. %) | | (atomic) | (%) | Site |
| **M-9-male** | −23.4 | −17.7 | +5.7 | +3.2 | +24.7 | 40.5 | 14.4 | 3.3 | 14.3 | **WH** |
| **M-10-male** | −23.3 | −18.3 | +5.0 | +2.4 | +25.6 | 41.1 | 14.6 | 3.3 | 16.7 | **WH** |
| **M-11-male** | −23.5 | −17.9 | +5.6 | +1.8 | +25.8 | 39.9 | 14.1 | 3.3 | 16.9 | **WH** |
| **M-12-male** | −23.9 | −18.9 | +5.0 | +2.0 | +22.3 | 42.2 | 15.0 | 3.3 | 15.3 | **WH** |
| **M-14** | −21.2 | −9.6 | +11.6 | +1.8 | +17.4 | 43.9 | 15.6 | 3.3 | 7.0 | **KL** |
| **M-15** | −22.7 | −8.9 | +13.8 | +1.8 | +15.8 | 41.8 | 14.3 | 3.4 | 12.9 | **KL** |
| **M-16** | −21.4 | **−8.8** | +12.6 | +1.9 | +18.8 | 36.2 | 12.8 | 3.3 | 8.9 | **KL** |

**$\delta^{13}C_{Col}$: $\delta^{13}C$ of bone collagen.**
**$\delta^{13}C_{Sc}$: $\delta^{13}C$ of bone bioapatite structural carbonate.**
**$\Delta^{13}C_{Sc\text{-}Col}$: C isotopic spacing between bone bioapatite structural carbonate and collagen.**
**Values in boldface font denote average of duplicates.**
**Yield: Collagen extraction yield.**
**WH: Whitehorse, International Eric Neilson airport.**
**KL: Eastern shoreline of Kluane Lake.**
***Unreliable owing to small sample size.**

**Table 3-8: CI and C/P values for fossil and modern bones (calculated using FTIR spectra).**

| Bone ID | Sampling Site | CI | C/P |
|---|---|---|---|
| | **Fossil Bones** | | |
| IC-9-2 | IC | 2.5 | 0.7 |
| IC-9-1 | IC | 2.5 | 0.9 |
| IC-19 | IC | 2.5 | 0.7 |
| GC-GZ-3-1 | GC | 2.5 | 0.7 |
| IC-14 | IC | 2.4 | 0.6 |
| QC-4 | QC | 2.8 | 0.6 |
| LB-GZ-1 | LB | 2.7 | 0.6 |
| IC-3 | IC | 2.8 | 0.6 |
| | **Modern Bones** | | |
| M-1-female | WH | 2.6 | 0.7 |
| M-2-male | WH | 2.5 | 0.6 |
| M-3-male | WH | 2.5 | 0.7 |
| M-5-male | WH | 2.5 | 0.7 |
| M-6-male | WH | 2.6 | 0.6 |
| M-7-male | WH | 2.5 | 0.7 |
| M-8-female | WH | 2.5 | 0.8 |
| M-9-male | WH | 2.5 | 0.7 |
| M-10-male | WH | 2.5 | 0.7 |
| M-11-male | WH | 2.7 | 0.6 |
| M-12-male | WH | 2.4 | 0.7 |
| M-14 | KL | 2.4 | 0.7 |
| M-15 | KL | 2.6 | 0.6 |
| M-16 | KL | 2.6 | 0.5 |

**WH: Whitehorse, International Eric Neilson airport.**
**KL: Eastern shoreline of Kluane Lake.**
**IC: Independence Creek; GC: Glacier Creek.**
**QC: Quartz Creek; LB: Little Blanche Creek.**

**Table 3-9: The $\delta^{18}O$ of structural carbonate and drinking water for bones.**

| Fossil Bone ID | Sampling Site | $\delta^{18}O_{SC}$ | $\delta^{18}O_p$ [1] | $\delta^{18}O_{dw}$ [2] |
|---|---|---|---|---|
| | | (‰, VSMOW) | | |
| **QC-4** | **QC** | +10.9 | +2.2 | −32.0 |
| **IC-3** | **IC** | +11.0 | +2.3 | −31.8 |
| **IC-9-1** | **IC** | **+13.4** | +4.6 | −27.0 |
| **IC-9-2** | **IC** | **+13.4** | +4.6 | −27.0 |
| **IC-14** | **IC** | +12.0 | +3.3 | −29.8 |
| **IC-19** | **IC** | +14.6 | +5.8 | −24.7 |
| **LB-GZ-1** | **LB** | +10.9 | +2.2 | −32.0 |
| **GC-GZ-3-1** | **GC** | **+12.7** | +4.0 | −28.4 |
| **Modern Bone ID** | | | | |
| **M-1-female** | **WH** | +24.1 | +15.1 | −5.6 |
| **M-2-male** | **WH** | **+25.0** | +16.0 | −3.8 |
| **M-3-male** | **WH** | +26.2 | +17.2 | −1.4 |
| **M-5-male** | **WH** | +27.4 | +18.4 | +1.0 |
| **M-6-male** | **WH** | +25.4 | +16.4 | −3.0 |
| **M-7-male** | **WH** | +25.1 | +16.1 | −3.6 |
| **M-8-female** | **WH** | +24.0 | +15.0 | −5.8 |
| **M-9-male** | **WH** | +24.7 | +15.7 | −4.4 |
| **M-10-male** | **WH** | +25.6 | +16.6 | −2.6 |
| **M-11-male** | **WH** | +25.8 | +16.8 | −2.2 |
| **M-12-male** | **WH** | +22.3 | +13.4 | −9.2 |
| **M-14** | **KL** | +17.4 | +8.6 | −19.0 |
| **M-15** | **KL** | +15.8 | +7.0 | −22.2 |
| **M-16** | **KL** | +18.8 | +9.9 | −16.3 |

**[1]: Calculated using Equation 3.2.**
**[2]: Calculated using Equation 3.3.**
**Values in boldface font denote average of duplicates.**
**WH: Whitehorse, International Eric Neilson airport; KL: Eastern shoreline of Kluane Lake; IC: Independence Creek; GC: Glacier Creek; QC: Quartz Creek; LB: Little Blanche Creek.**

### 3.4.7 $^{13}C$ and $^{15}N$ Enrichment of Ground Squirrel Bone Collagen relative to Plants in Modern Study Areas

The average $^{13}C$ and $^{15}N$ enrichments from bulk plant to ground squirrel bone collagen have been calculated using the average $\delta^{13}C$ and $\delta^{15}N$ of modern plants (Chapter 2) and bones collected from the Yukon (Figs. 3-10, 3-11): +2.0 and +1.9 ‰ for $^{15}N$, and +4.2 ‰ and +5.2 ‰ for $^{13}C$ for Whitehorse and Kluane Lake, respectively. The calculated $\Delta^{13}C_{Col\text{-}diet}$ and $\Delta^{15}N_{Col\text{-}diet}$ are in the range already reported for both small and large mammals in different experimental conditions (N: Ambrose, 2000; DeNiro and Epstein, 1981; Hare et al., 1991; Vogel et al., 1990, C: DeNiro and Epstein, 1981; Howland et al., 2003; Jim et al., 2004; Vogel, 1978).

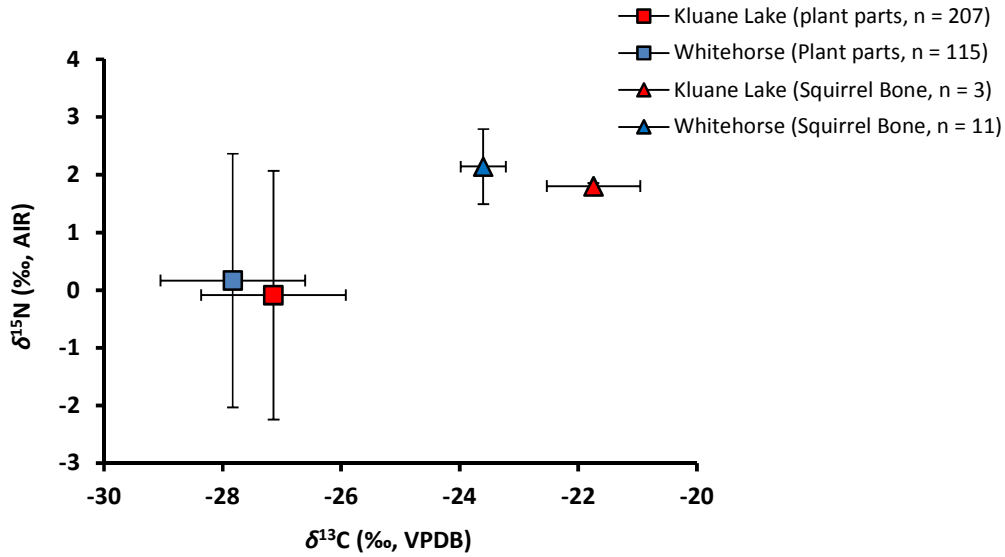


**Figure 3-10: Average $\delta^{13}C$ and $\delta^{15}N$ of modern plants and ground squirrel bone collagen from Kluane Lake and Whitehorse.**

Different factors have been suggested to affect $^{13}C$ and $^{15}N$ spacing between diet and consumer's collagen including macromolecular composition of diet, species (DeNiro and Epstein, 1981; Hobson and Clark, 1992; Sponheimer et al., 2003), physiology (Ambrose, 1990; Sponheimer et al., 2003), environment (hot and arid *vs.* cool and wet) (Ambrose, 1990) and age (Hobson and Clark, 1992). Some of these factors could contribute in the small difference observed in $^{13}C$ and $^{15}N$ spacing values between two study sites.

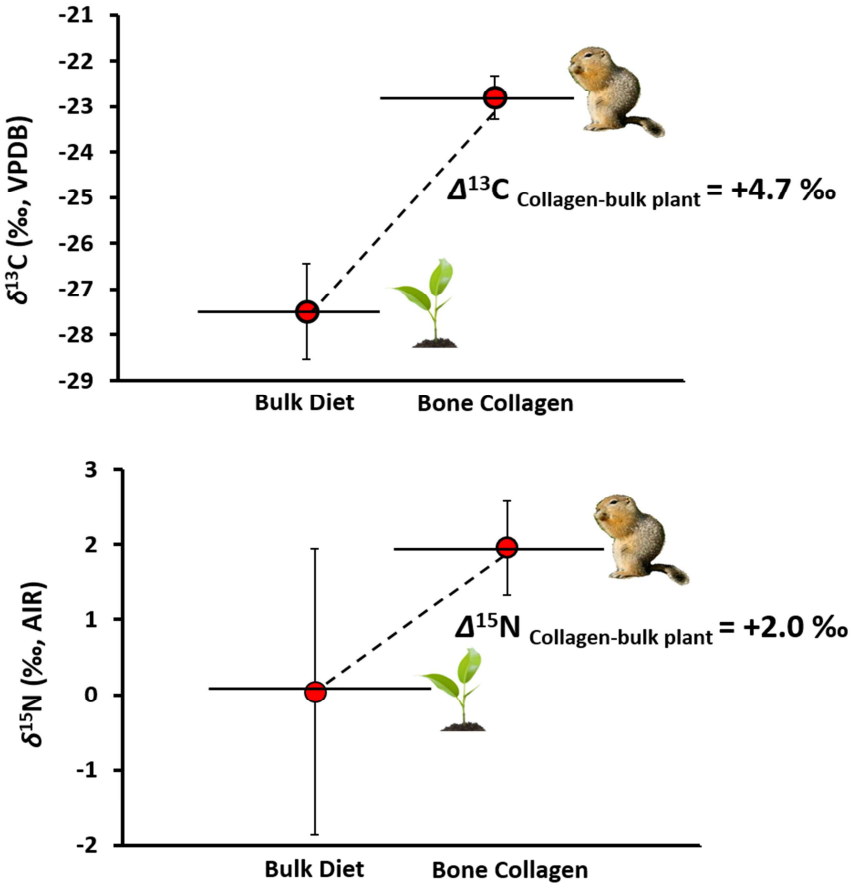


**Figure 3-11: Average $^{13}C$ and $^{15}N$ enrichment from bulk plant to ground squirrels bone collagen from Kluane Lake and Whitehorse.**

## 3.4.8 Modern Plant Decomposition Experiment

### 3.4.8.1 Site Weather Data

Table 3-10 presents the monthly mean temperature and total precipitation for the Environment Canada weather station closest (~8 km) to the site of decomposition experiment. The first interval (October 21, 2013 to April 2, 2014) received the highest total amount of precipitation (388.2 mm) and experienced the lowest mean temperature (–2.7°C). The second interval (April 2, 2014 to June 30, 2014) received 254.2 mm total precipitation with mean temperature of +13.3°C. For the last interval (June 30, 2014 to September 2, 2014), total precipitation and mean temperature were 318.5 mm and +18.0°C, respectively.

**Table 3-10: Weather data for London, ON, Canada.**

| Month | MAT (°C)[1] | TMP (mm)[2] |
|---|---|---|
| | **2013**[3] | |
| **October** | +11.5 | 155.3 |
| **November** | +1.6 | 51.4 |
| **December** | −4.5 | 57.1 |
| | **2014**[3] | |
| **January** | −9.2 | 49.6 |
| **February** | −10.2 | 49.5 |
| **March** | −5.1 | 25.3 |
| **April** | +6.3 | 76.2 |
| **May** | +13.8 | 81.8 |
| **June** | +19.7 | 96.2 |
| **July** | +18.8 | 109.7 |
| **August** | +19.3 | 43.8 |
| **September** | +16.0 | 165.0 |

[1] **MAT**: Mean Air Temperature (data from Environment Canada, 2015)
[2] **TMP**: Total Monthly Precipitation (data from Environment Canada, 2015)
[3] Data from London CS station (43°02'00.000" N, 81°09'00.000" W)

### 3.4.8.2 Isotopic Data

Figure 3-12a,b ($\delta^{13}$C) and Figure 3-13a,b ($\delta^{15}$N) illustrate and Table 3-11 lists the average $\delta^{13}$C and $\delta^{15}$N of decomposed plants at each of 4 sampling times over the 317 days of the decomposition experiment. All data including replicates (3 for each sampling time) are presented in Appendix H. Table 3-12 summarizes the results of repeated measure ANOVA testing for the time effect on plant $\delta^{13}$C and $\delta^{15}$N over the course of this experiment, and for samples showing a significant time dependence on their isotopic compositions, the results of the Benferroni *post hoc* test are summarized in Tables 3-13 ($\delta^{13}$C) and 3-14 ($\delta^{15}$N).

All plant species used in this experiment had initial $\delta^{13}$C ranging from –29.5 to –25.6 ‰. *C. purpurascens* is the only plant species showing a significant shift in $\delta^{13}$C while buried in soil over 317 days, changing from –27.5 ‰ at Day 1 to –25.5 ‰ at Day 317 (Table 3-11). Significant $^{13}$C-enrichment occurred only during the first 164 days (Table 3-13). Other plant species show some smaller changes in $\delta^{13}$C with time during both the 'buried' and 'not buried' treatments, but these are not statistically significant. This may indicate heterogeneity in the samples, as suggested by the high SD of average $\delta^{13}$C at each sampling time (Table 3-11), and/or an insufficiently long period of time for the effects of decomposition to become significant.

Greater change is observed in plant $\delta^{15}$N during the decomposition experiment. Over the course of the burial treatment, $^{15}$N-enrichment ranges from 2.4 ‰ for *P. glauca* to 10.0 ‰ for *F. altaica* (Table 3-11). This change is statistically significant for all species except *E. spicatus* (Table 3-12). All samples show the same pattern of $^{15}$N-enrichment from Day 1 to Day 317. The first time interval (164 days) showed the most $^{15}$N-enrichment for all samples, followed by fluctuations in nitrogen isotopic composition to the end of the experiment (317 days) (Fig. 3-13a).

The measured change in $\delta^{15}$N during the decomposition experiment for the 'not buried' samples is very different. *P. glauca* shows a negative shift in $\delta^{15}$N to the end of the experiment (Table 3-12) with the most significant changes occurring between Days 1 and 164 and Days 1 and 317 (Table 3-14). The changes in nitrogen isotopic composition with time (Fig. 3-13b) measured for the other species are not statistically significant (Table 3-12).

**Table 3-11: Average $\delta^{13}C$ and $\delta^{15}N$ of plant detritus during decomposition.**

| Plant | Time | $\delta^{13}C$ (‰, VPDB) | | $\delta^{15}N$ (‰, AIR) | |
|---|---|---|---|---|---|
| species | (days) | Buried | Not buried | Buried | Not buried |
| *E. trachycaulus* | 1 | −27.7 (± 0.1)* | −27.7 (± 0.1) | −1.0 (± 1.7) | −1.0 (± 1.7) |
| | 164 | −27.5 (± 0.7) | −28.4 (± 0.3) | +5.3 (± 1.9) | −0.2 (± 1.2) |
| | 253 | −28.6 (± 0.6) | −27.6 (± 0.7) | +4.5 (± 0.6) | −1.1 (± 0.7) |
| | 317 | −27.9 (± 0.8) | −27.4 (± 0.5) | +2.3 (± 1.3) | −2.8 (± 1.1) |
| *C. purpurascens* | 1 | −27.5 (± 0.1) | −27.5 (± 0.1) | −0.8 (± 0.5) | −0.8 (± 0.1) |
| | 164 | −24.4 (± 0.1) | −24.9 (± 0.3) | +3.2 (± 0.7) | −1.3 (± 0.8) |
| | 253 | −24.6 (± 0.8) | −25.1 (± 0.4) | +2.9 (± 0.5) | −1.3 (± 3.1) |
| | 317 | −25.5 (± 0.7) | −24.8 (± 1.0) | +2.9 (± 0.6) | −1.6 (± 0.8) |
| *P. glauca* | 1 | −25.6 (± 0.7) | −25.6 (± 0.7) | +0.6 (± 0.2) | +0.6 (± 0.2) |
| | 164 | −26.3 (± 0.3) | −26.7 (± 0.2) | +1.2 (± 1.1) | −0.9 (± 0.4) |
| | 253 | −26.7 (± 0.4) | −26.9 (± 0.6) | +2.6 (± 0.9) | −2.5 (± 1.0) |
| | 317 | −27.2 (± 0.6) | −27.1 (± 0.1) | +3.1 (± 0.1) | −3.1 (± 0.6) |
| *F. altaica* | 1 | −27.6 (± 0.1) | −27.6 (± 0.1) | −4.7 (± 3.4) | −4.7 (± 3.4) |
| | 164 | −27.3 (± 0.2) | −27.7 (± 0.3) | +3.7 (± 2.8) | −0.2 (± 1.4) |
| | 253 | −28.0 (± 0.3) | −27.4 (± 0.2) | +6.5 (± 0.9) | −1.0 (± 2.8) |
| | 317 | −27.4 (± 0.7) | −26.7 (± 1.1) | +5.3 (± 0.3) | −1.7 (± 0.2) |
| *A. frigida* | 1 | −29.5 (± 0.6) | −29.5 (± 0.6) | −2.4 (± 1.2) | −2.4 (± 1.2) |
| | 164 | −30.7 (± 0.2) | −29.4 (± 0.1) | −0.8 (± 0.3) | −2.0 (± 0.3) |
| | 253 | −30.2 (± 0.9) | −29.7 (± 0.3) | +0.9 (± 0.3) | +0.2 (± 1.8) |
| | 317 | −30.7 (± 0.4) | −29.2 (± 0.4) | +0.2 (± 0.3) | −1.2 (± 0.3) |
| *E. spicatus* | 1 | −27.1 (± 0.3) | −27.1 (± 0.3) | −3.1 (± 1.4) | −3.1 (± 1.4) |
| | 164 | −27.4 (± 0.7) | −27.1 (± 0.1) | +4.0 (± 0.3) | −1.9 (± 1.4) |
| | 253 | −27.3 (± 1.7) | −27.6 (± 1.0) | +4.3 (± 3.3) | −3.4 (± 1.6) |
| | 317 | −27.2 (± 0.4) | −27.5 (± 0.6) | +4.1 (± 0.3) | −4.2 (± 1.2) |

***Values in parentheses are SD.**

**Table 3-12: *p*-value results of repeated measure ANOVA (Greenhouse-Geisser) test for time-dependent effects on $\delta^{13}C$ and $\delta^{15}N$ of plant detritus.**

| Plant ID | Treatments | *p*-value | |
|---|---|---|---|
| | | $\delta^{13}C$ (‰, VPDB) | $\delta^{15}N$ (‰, AIR) |
| ***E. trachycaulus*** | **B** | 0.210 | **0.042** |
| | **NB** | 0.241 | 0.256 |
| ***C. purpurascens*** | **B** | **0.021** | **0.008** |
| | **NB** | 0.052 | 0.795 |
| ***P. glauca*** | **B** | 0.148 | **0.034** |
| | **NB** | 0.131 | **0.004** |
| ***F. altaica*** | **B** | 0.351 | **0.012** |
| | **NB** | 0.316 | 0.327 |
| ***A. frigida*** | **B** | 0.230 | **0.050** |
| | **NB** | 0.480 | 0.227 |
| ***E. spicatus*** | **B** | 0.842 | 0.079 |
| | **NB** | 0.589 | 0.371 |

**B: Buried NB: Not Buried**

**Values in boldface font are statistically significant ($p \leq 0.05$).**

**Table 3-13: *p*-value results of Benferroni *post hoc* test for differences in average $\delta^{13}C$ between Day 1 and days 164, 253 and 317 for samples showing significant time-dependent effect after the repeated measure ANOVA (Greenhouse-Geisser) test.**

| Plant ID | B-1 to B-164 | B-1 to B-253 | B-1 to B-317 |
|---|---|---|---|
| | $\delta^{13}C$ (‰, VPDB) | | |
| *C. purpurascens* | **0.010** | 0.203 | 0.256 |

**B: Buried 1: Day 1 253: Day 253 317: Day 317**
**Values in boldface font are statistically significant ($p \leq 0.05$).**

**Table 3-14: *p*-value results of Benferroni *post hoc* test for differences in average $\delta^{15}N$ between Day 1 and days 164, 253 and 317 for samples showing significant time effect after the repeated measure ANOVA (Greenhouse-Geisser) test.**

| Plant ID | B-1 to B-164 | B-1 to B-253 | B-1 to B-317 | NB-1 to NB-164 | NB-1 to NB-253 | NB-1 to NB-317 |
|---|---|---|---|---|---|---|
| | $\delta^{15}N$ (‰, AIR) | | | | | |
| *E. trachycaulus* | **0.080** | 0.317 | 0.551 | - | - | - |
| *C. purpurascens* | 0.073 | **0.034** | 0.173 | - | - | - |
| *P. glauca* | 1.000 | 0.513 | **0.015*** | - | - | - |
| | - | | - | **0.031** | 0.145 | **0.066** |
| *F. altaica* | 0.317 | 0.149 | 0.240 | - | - | - |
| *A. frigida* | 1.000 | 0.136 | 0.210 | - | - | - |

**B: Buried NB: Not Buried 1: Day 1 253: Day 253 317: Day 317**
**Values in boldface font are statistically significant ($p \leq 0.05$).**

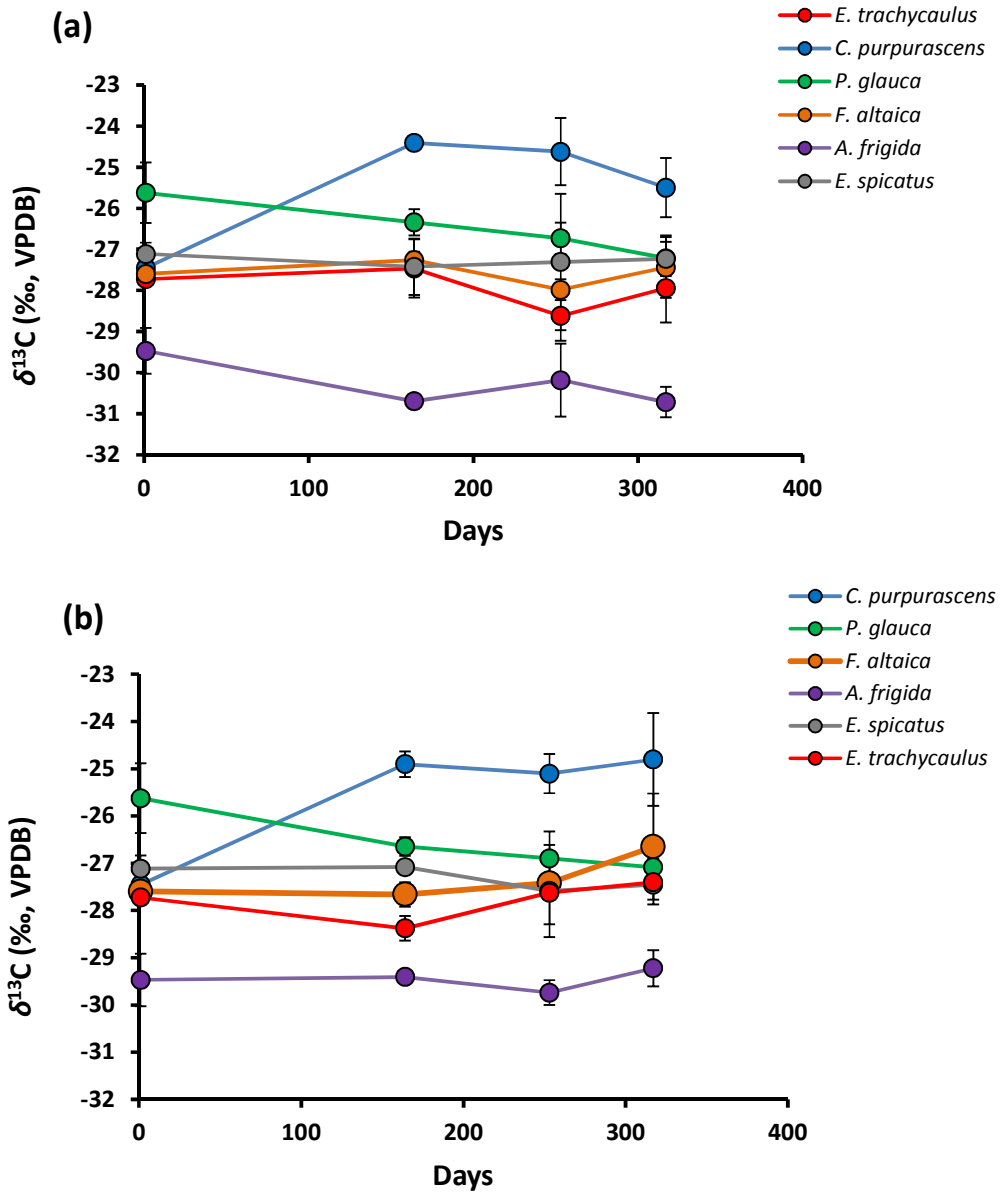


**Figure 3-12: Variation in plant $\delta^{13}$C during decomposition under (a) 'buried', and (b) 'not buried' conditions.**

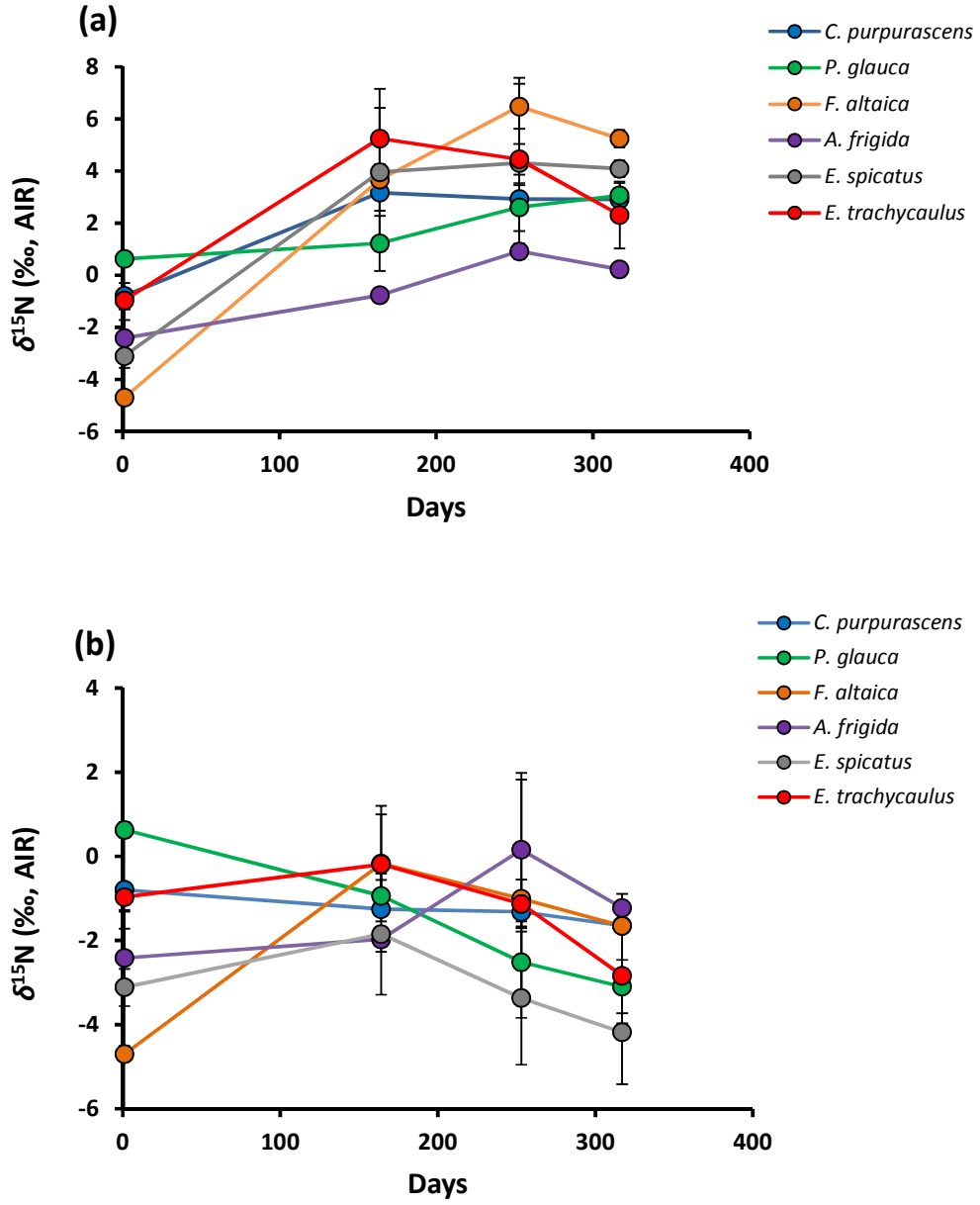


**Figure 3-13: Variation in plant $\delta^{15}$N during decomposition under (a) 'buried', and (b) 'not buried' conditions.**

### 3.4.8.3 C and N contents and atomic C/N

Average C and N contents and atomic C/N of samples from the decomposition experiment are presented in Table 3-15. The complete dataset, including replicates, is presented in Appendix H. The initial C and N contents (wt. %) of all samples range from 40 to 45 % and 0.5 to 1.4 %, respectively. The atomic C/N of the starting materials has a

wide range (36.9 to 105.2), with *P. glauca* and *A. frigida* having the lowest ratio (< 50) mainly due to their higher N content than the other plant species.

During the burial experiment, the atomic C/N of all species decrease, for except *A. frigida* (Fig. 3-14a), with *P. glauca* and *E. spicatus* showing the smallest (16.3) and largest (77.7) change, respectively. There is a significant positive correlation between atomic C/N and C (wt. %) (R = 0.551, *p*-value = 0.005) for all species over the course of the burial experiment, which shows that the change in atomic C/N over 317 days is driven mainly by the change in C content. *A. frigida* is the only sample showing a final slight increase in atomic C/N, along with lower N and C contents, relative to the starting composition. The N reduction most probably caused its opposite pattern in atomic C/N shift. In general, the change in atomic C/N occurs in three main steps: (i) A change in C/N in all species (from a wide range of 35-110) to a narrower range of 50-60 by Day 164, (ii) a continuous lowering of C/N to 20 to 34 between Days 164 and 253, and (iii) only minor variation in C/N between Days 253 and 317 (Fig. 3-14a).

For 'not buried' samples, only *C. purpurascens, F. altaica* and *E. spicatus* exhibit a decrease in atomic C/N (Fig. 3-14b), with a reduction in C content and almost no change in N content occurring over the course of the experiment (Table 3-15) . *E. trachycaulus, A. frigida* and *P. glauca,* by comparison, show a small to negligible increase in atomic C/N. Both C and N contents decrease in *E. trachycaulus*, while *A. frigida* shows only decrease in N content over the 317 days of decomposition experiment (Fig. 3-14b).

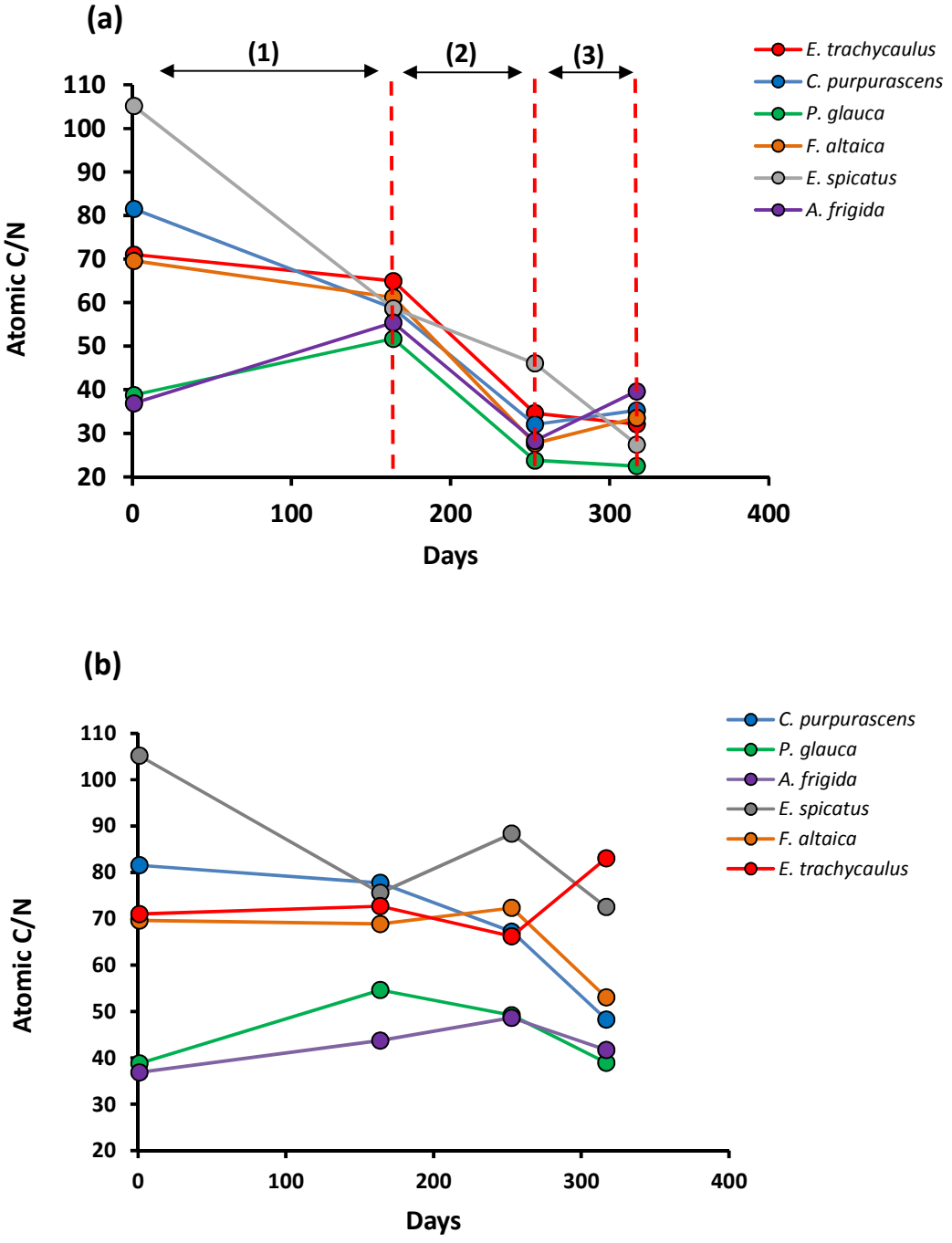


**Figure 3-14: Variation in atomic C/N during decomposition under (a) 'buried' and (b) 'not buried' conditions.**

**Table 3-15: Average C and N contents and atomic C/N of plant detritus during decomposition.**

| Plant | Time | C (wt. %) | | N (wt. %) | | Atomic C/N | |
|---|---|---|---|---|---|---|---|
| species | (days) | Buried | Not buried | Buried | Not buried | Buried | Not buried |
| ***E. trachycaulus*** | **1** | 43.0 (± 1.2)* | 43.0 (± 1.2) | 0.7 (± 0.3) | 0.7 (± 0.3) | 71.0 | 71.0 |
| | **164** | 40.9 (± 2.0) | 41.4 (± 1.6) | 0.7 (± 0.3) | 0.7 (± 0.4) | 65.0 | 72.7 |
| | **253** | 37.5 (± 5.5) | 39.5 (± 6.3) | 1.3 (± 0.1) | 0.7 (± 0.3) | 34.6 | 66.2 |
| | **317** | 37.9 (± 3.5) | 38.5 (± 1.0) | 1.4 (± 0.2) | 0.5 (± 0.1) | 32.1 | 83.0 |
| ***C. purpurascens*** | **1** | 41.0 (± 2.0) | 41.0 (± 2.0) | 0.6 (± 0.1) | 0.6 (± 0.1) | 81.5 | 81.5 |
| | **164** | 39.3 (± 2.0) | 38.4 (± 1.3) | 0.8 (± 0.1) | 0.6 (± 0.1) | 58.7 | 77.7 |
| | **253** | 34.0 (± 3.7) | 38.1 (± 5.0) | 1.2 (± 0.3) | 0.7 (± 0.3) | 32.0 | 67.2 |
| | **317** | 36.2 (± 4.4) | 32.7 (± 2.9) | 1.2 (± 0.1) | 0.8 (± 0.1) | 35.2 | 48.3 |
| ***P. glauca*** | **1** | 41.0 (± 0.4) | 41.0 (± 0.4) | 1.2 (± 0.3) | 1.2 (± 0.3) | 38.8 | 38.8 |
| | **164** | 39.9 (± 0.9) | 40.6 (± 2.0) | 0.9 (± 0.1) | 0.9 (± 0.2) | 51.7 | 54.6 |
| | **253** | 28.2 (± 3.2) | 34.5 (± 5.3) | 1.4 (± 0.2) | 0.8 (± 0.1) | 23.7 | 49.2 |
| | **317** | 29.3 (± 0.9) | 30.8 (± 1.4) | 1.5 (± 0.0) | 0.9 (± 0.1) | 22.5 | 39.0 |
| ***F. altaica*** | **1** | 41.0 (± 1.8) | 41.0 (± 1.8) | 0.7 (± 0.3) | 0.7 (± 0.3) | 69.6 | 69.6 |
| | **164** | 33.6 (± 5.0) | 34.7 (± 2.9) | 0.6 (± 0.2) | 0.6 (± 0.2) | 61.2 | 68.9 |
| | **253** | 31.8 (± 3.9) | 34.3 (± 9.0) | 1.3 (± 0.4) | 0.6 (± 0.2) | 27.7 | 72.3 |
| | **317** | 32.2 (± 3.4) | 30.3 (± 7.4) | 1.1 (± 0.16) | 0.7 (± 0.1) | 33.5 | 53.0 |
| ***A. frigida*** | **1** | 44.9 (± 0.9) | 44.9 (± 0.9) | 1.4 (± 0.1) | 1.4 (±0.1) | 36.9 | 36.9 |
| | **164** | 47.2 (± 0.9) | 46.3 (± 2.3) | 1.0 (± 0.1) | 1.2 (±0.1) | 55.4 | 43.7 |
| | **253** | 42.6 (± 1.6) | 46.1 (± 3.6) | 1.8 (± 0.4) | 1.1 (± 0.1) | 28.3 | 48.6 |

**Table 3-15 Cont'd.**

| Plant | Time | C (wt. %) | | N (wt. %) | | Atomic C/N | |
|---|---|---|---|---|---|---|---|
| species | (days) | Buried | Not buried | Buried | Not buried | Buried | Not buried |
| | **317** | 42.7 (± 3.4) | 44.5 (± 1.4) | 1.3 (± 0.2) | 1.2 (± 0.1) | 39.6 | 41.7 |
| ***E. spicatus*** | **1** | 43.0 (± 1.7) | 43.0 (± 1.7) | 0.5 (± 0.3) | 0.5 (± 0.3) | 105.2 | 105.2 |
| | **164** | 41.3 (± 1.9) | 40.2 (± 1.1) | 0.8 (± 0.1) | 0.6 (± 0.1) | 58.7 | 75.6 |
| | **253** | 35.6 (± 4.8) | 39.1 (± 1.9) | 0.9 (± 0.5) | 0.5 (± 0.1) | 46.1 | 88.3 |
| | **317** | 26.1 (± 3.1) | 37.5 (± 1.5) | 1.1 (± 0.1) | 0.6 (± 0.1) | 27.4 | 72.5 |

***Values in parentheses are SD.**

## 3.5 Discussion

The results of the decomposition experiment are discussed first because the potential influence of decay process on the isotopic composition of plant macrofossils should be considered in interpretation of the fossil plant results.

### 3.5.1 Modern Plant Decomposition Experiment

The C isotopic composition of plant litter ($\delta^{13}C_{litter}$) does not show a clear pattern of increasing or decreasing with increasing time of decomposition. In the 'buried' treatment, *C. purpurascens* is the only species showing a significant enrichment in $^{13}C$ with time, particularly within the first interval (164 days). Such $^{13}C$ enrichment has been reported previously in litter decomposition studies (Gleixner et al., 1993) and for OM downwards in soil profiles (Boström et al., 2007; Krull et al., 2002; Wang et al., 2008; Wynn, 2006, 2007).

There are two main processes that can change $\delta^{13}C_{litter}$ (Ågren et al., 1996; Connina et al., 2001): (i) a continuous change in the quality and chemical composition of decomposing debris, which should decrease $\delta^{13}C_{litter}$ over time due to the loss of $^{13}C$-enriched compounds such as cellulose, sugar and starch in the early stages of alteration, leaving more $^{13}C$-depleted and recalcitrant compounds such as lignin in the later stages of decomposition (Benner et al., 1987; Gleixner et al., 1993; Hedges et al., 1985; Lichtfouse et al., 1995; Minderman, 1968; Opsahl and Benner, 1995), and (ii) isotopic effects from decomposers (microbes) either through discrimination during plant decomposition or direct biomass contribution, which increases $\delta^{13}C_{litter}$ over time (Dijkstra et al., 2006; Lichtfouse et al., 1995). The second process can best account for the observed $^{13}C$ enrichment of *C. purpurascens* during the first 164 days of the decomposition experiment. A change in substrate quality as the decomposition progressed could change the balance between these two processes, causing the change in pattern for $\delta^{13}C_{litter}$ observed for *C. purpurascens* before and after Day 164 (Fig. 3-12a).

Relative to carbon, the nitrogen isotopic behavior of the plant tissue is quite different. Microscopic images illustrate the change in plant debris color and texture: (i) before decomposition, (ii) after decomposition on soil surface, and (iii) after decomposition in soil (Fig. 3-15). The decomposed 'buried' plants have a much darker color and show greater modification of their tissues than the other samples. The 'buried' plant detritus also have higher abundances of microbial colonies (fungal hypha) on the surface (Fig. 3-16). Considering these images and much larger shift in $\delta^{15}$N and C/N of 'buried' samples (Figs. 3-13a, 3-14a), we conclude that the magnitude of decomposition was greater for the 'buried' than 'not buried' samples. This is expressed in particular by the significant $^{15}$N-enrichment (2.5-10 ‰) over 317 days for all species in the 'buried' experiment; except for *E. spicatus* (where the difference was not statistically significant) (Table 3-12).

While such a change is consistent with several previous studies (Kramer et al., 2003; Krull et al., 2002; Tremblay and Benner, 2006), it does not support the inhibitory role of soil particles on decomposition suggested by Veen and Kuikman (1990). Greater decay of 'buried' *vs*. 'not buried' plant detritus could be explained by: (i) a buffering role of soil in balancing water and oxygen availability and temperature for decomposers (Herman et al., 1976), and/or (ii) the activity of earthworms in soil. Moisture content has a strong influence on microbial populations (Campbell and Biederbeck, 1976). Soil water content undergoes greater fluctuations at the soil surface because of direct exposure to wind, light and rain than deeper in the soil (2 cm), which comprises a more stable environment and a better balance of moisture and oxygen for microbes than the soil surface. A lower rate of plant decomposition in the absence of soil has been reported previously (Herman et al., 1976). Abundant earthworms were also observed around the 'buried' plant detritus at the time of the 253 and 317 days' sampling (Fig. 3-17). As discussed earlier in section 3.1.4.1, the important role of earthworms in accelerating the rate of OM decomposition and nutrient cycling has been reported in many studies (Singh and Gupta, 1977; Spiers et al., 1986; Wardle, 2002).

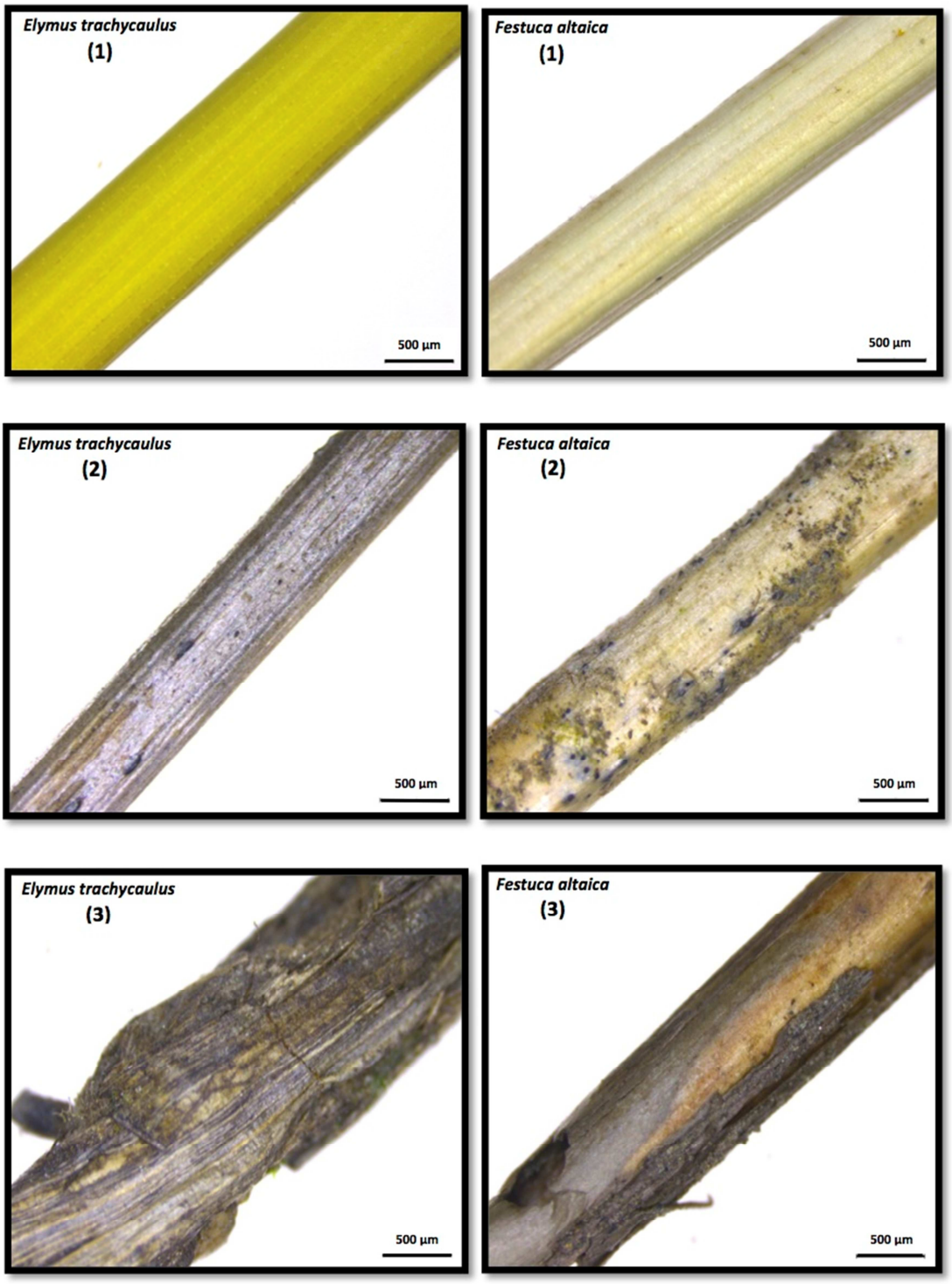


**Figure 3-15: Images of *E. trachycaulus and F. altaica*: (1) before decomposition, (2) after decomposition on soil surface, and (3) after decomposition in soil.**

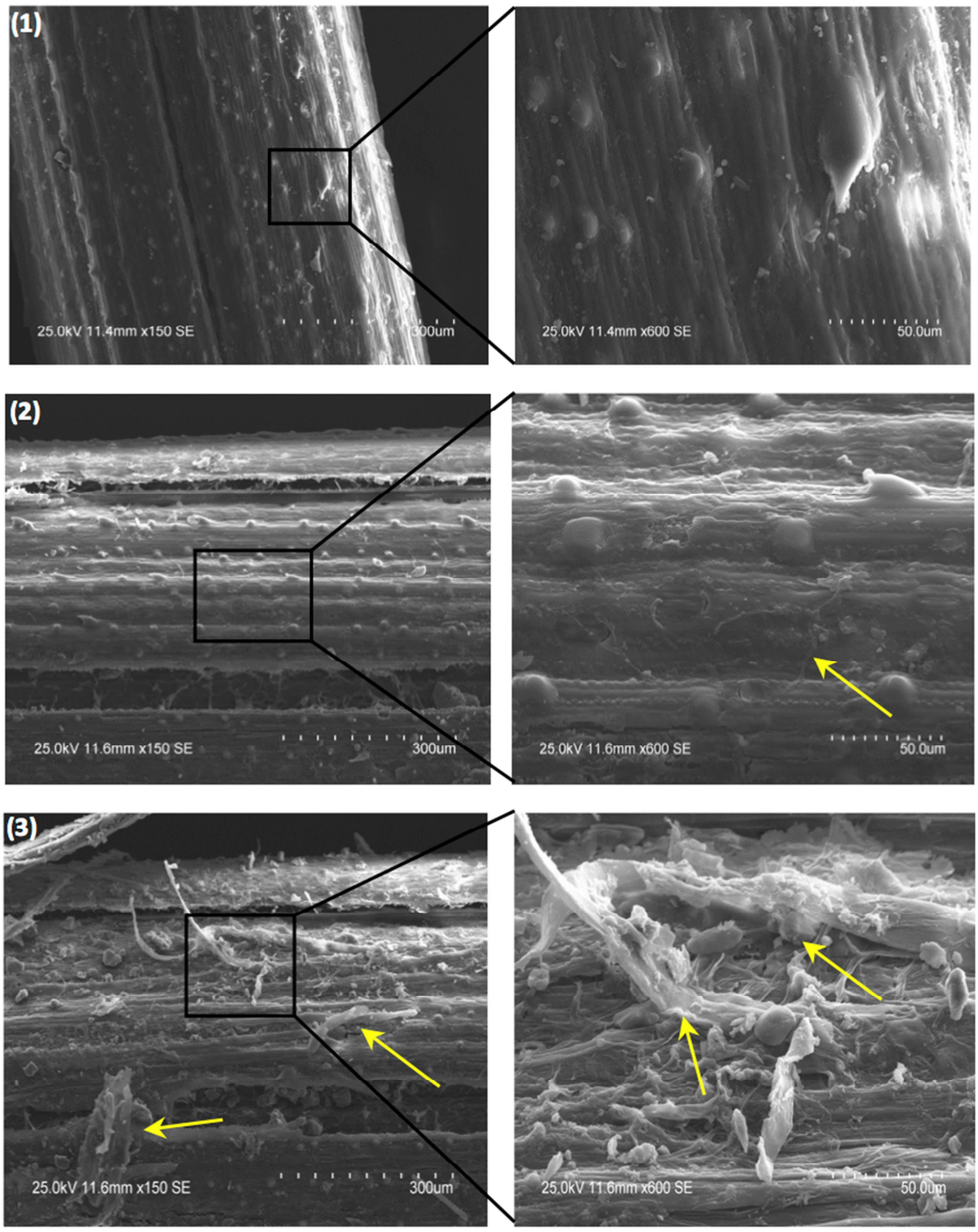


**Figure 3-16: SEM images of *F. altaica*: (1) before decomposition, (2) after decomposition on soil surface, and (3) after decomposition in soil. (Yellow arrows: fungal hypha).**

**Figure 3-17: Earthworms (red arrows) and their casts (yellow arrows) in vicinity of 'buried' plant detritus.**

Most of the $^{15}$N-enrichment occurred in the 'buried' samples and during the first interval (first 164 days), particularly for *F. altaica*, *E. spicatus*, *E. trachycaulus* and *C. purpurascens*, and was accompanied by a larger decrease in atomic C/N (Figs. 3-13a, 3-14a). Lower $^{15}$N-enrichment and little change in atomic C/N were measured for *P. glauca* and *A. frigida*. During the first 164 days of the experiment, the samples received the highest amount of precipitation (Table 3-10) and also were exposed to microbially mediated decay under winter snow cover, which insulated the underlying environment from surface conditions. Two processes (leaching and microbially mediated decomposition) can be proposed to cause changes in $\delta^{15}$N and atomic C/N of the plant debris. The relative importance of these two processes depends on the initial chemical composition of the plant (Benner et al., 1990; Melin, 1930; Wang et al., 2004). The lower mean temperature of the first interval (Table 3-10) may have limited the rate of microbial decay relative to later intervals. The interplay of a longer time period, more total precipitation, the influence of winter snow cover in moderating soil temperature, and higher quality of plant debris at this first stage, however, appear to have facilitated greater decay during this period of decomposition.

Plants containing more N (*P. glauca*, *A. frigida*) likely contain more water-soluble N compounds and hence are more affected by leaching. Leaching causes the loss of some $^{15}$N-depleted compounds at early stages of decomposition, which in turn reduces the N

content and increases $\delta^{15}$N. These plants also experienced a reduction in C content associated with microbial decomposition, with the net effect of smaller changes in atomic C/N.

It appears that plants such as *F. altaica*, *E. spicatus*, *E. trachycaulus* and *C. purpurascens*, which have lower initial N contents than *P. glauca* and *A. frigida* are affected less by N-loss arising from leaching while experiencing C loss through microbial decomposition. This reduces their atomic C/N and increases their $\delta^{15}$N by contribution of $^{15}$N-enriched microbial products. Why *P. glauca* and *A. frigida* do not show greater $^{15}$N enrichment during the early stages may be related to leaf quality factors such as water soluble content (Wardle et al., 2003) and C quality (Hobbie, 1996). *Artemisia* essential oils have been shown to have an inhibitory effect on bacteria and fungi activities (Lopes-Lutz et al., 2008). In addition, a grassland study close to Kluane Lake showed *A. frigida* to have a lower rate of decomposition than *P. glauca* and a legume, perhaps because of anti-herbivory alkaloids or anti-fungal secondary metabolites (McLaren and Turkington, 2011; Talley et al., 2002).

After the first 164 day interval, there are some fluctuations in $\delta^{15}$N until Day 317 at which the final $\delta^{15}$N of the decomposed plant tissues are higher than the initial values. These fluctuations can be explained by continuous change in litter quality and environmental factors (temperature and moisture) and subsequently the population and activity of microbes and their interaction toward the end of experiment (Harvey et al., 1995). Bacteria populations in soil experience diurnal and even hourly fluctuations (Taylor, 1936; Thornton and Taylor, 1935). We have proposed the contribution of microbial biomass as one of the main factors causing the significant enrichment in $^{15}$N of the decomposed tissues during the first 164 days. The lower magnitude of $^{15}$N-enrichment during the last two stages probably reflects the lower quality of substrates, which slows down the rate of decomposition and microbial colonization.

A simple model for the processes that may contribute to $^{13}$C- and $^{15}$N-enrichment of the 'buried' plants is illustrated in Figure 3-18. Leaching during early stages of decomposition (Pardo et al., 1997; Salazar et al., 2012) leaves the system slightly

enriched in $^{15}N$ by removal of $^{15}N$-depleted compounds (Houlton and Bai, 2009). The importance of this process depends on the environment under which decomposition occurs and the water-soluble content of the plant litter. Microbial attack on plant C-compounds and microbial respiration releases $^{13}C$-depleted $CO_2$ that leaves microbial products enriched in $^{13}C$, and causes a gradual decrease in atomic C/N in decomposing plants.

A significant negative correlation exists between $\delta^{15}N$ and atomic C/N ($R = –0.515$, $p$-value = 0.010) and $\delta^{15}N$ and C (wt. %) ($R = –0.637$, $p$-value = 0.001) for all six species over 317 days (Fig. 3-19a,b). Higher $\delta^{15}N$ of decomposed plants is associated with lower atomic C/N and C content, suggesting (i) more C and N mineralization as plant decay proceeds, and (ii) reduction in atomic C/N driven mainly by C reduction. Carbon mineralization reduces C and atomic C/N, and lower C contents induce microbial N dissimilation (Dijkstra et al., 2008). Dijkstra et al. (2008) also found a negative correlation between microbial $^{15}N$-enrichment and soil-soluble C/N and a positive correlation between microbial $^{15}N$-enrichment and net N mineralization. They suggested that early stages of plant decay is characterized with more microbial assimilation of N and less release of $NH_3$, which is $^{15}N$-depleted, while in the late stages N dissimilation is prevalent and more $NH_3$, which is $^{15}N$-depleted, is released into the soil. Once labile C-compounds become limited because of microbial usage during early stages, microbes then attack N-compounds. Microbes incorporate some portions of organic N directly into their body and dissimilate other portions into $NH_4^+$. Depending on the N demand of the microbes some $NH_4^+$ is again assimilated by microbes and some is released back to the system as $NH_3$ (Fig. 3-18). The $NH_3$ then becomes available to plants and other microbes following conversion to $NH_4^+$ or is absorbed on clay minerals. N isotopic fractionation during all these microbial processes (dissimilation, Hogberg, 1997; assimilation, Robinson, 2001; $NH_4^+$ to $NH_3$ conversion, Hogberg, 1997) leads to $^{15}N$-enrichment of microbes and release of $^{15}N$-depleted $NH_3$ (Dijkstra et al., 2008). The effect of the gut contents of earthworms on the N isotopic composition of decomposing plants is unknown, but nutrient-rich casts of earthworms most probably add N to the system (Fig. 3-17), which in turn might compensate for N loss through leaching.

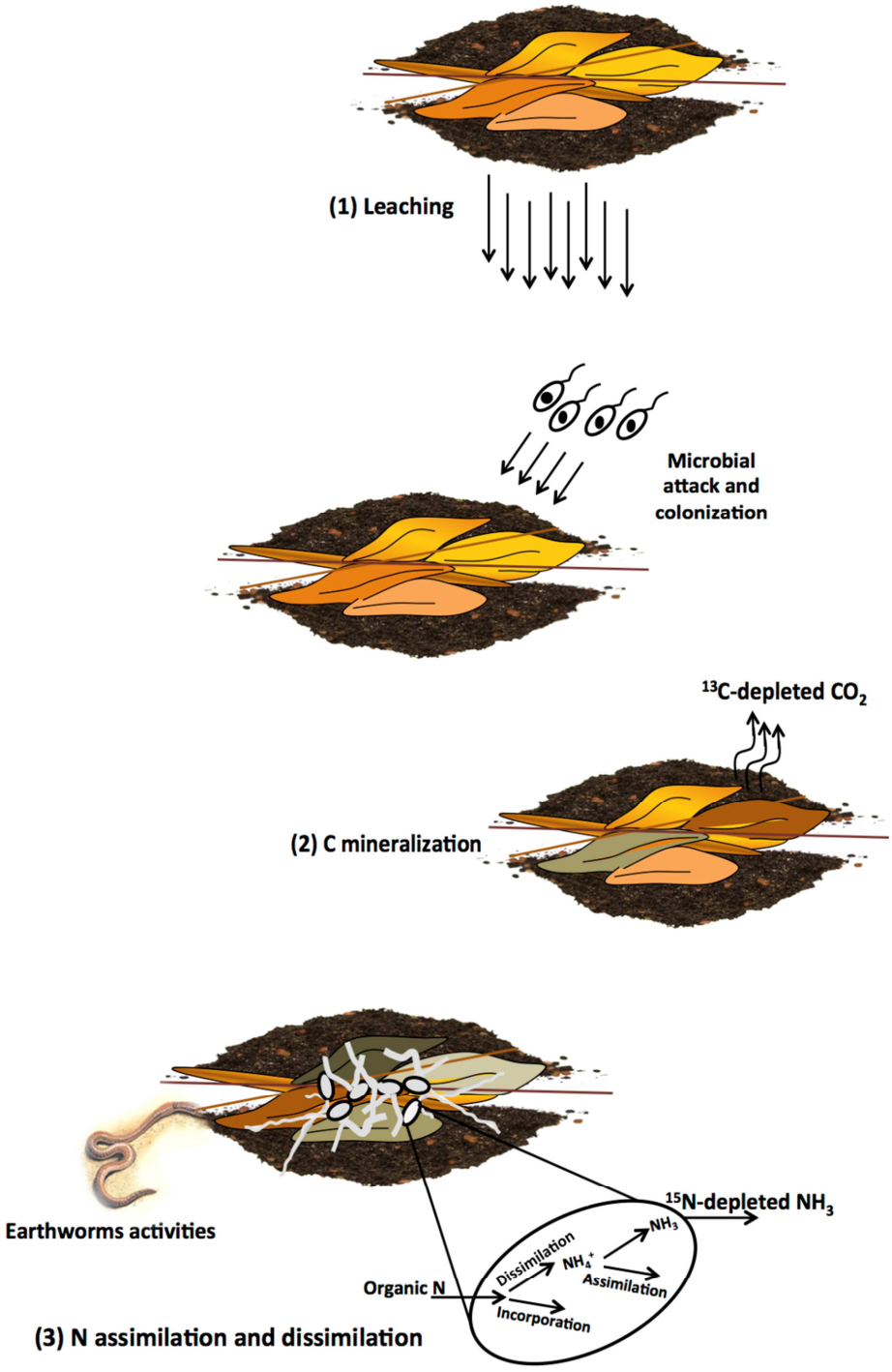


**Figure 3-18: Model for plant decay and main processes contributing to C and N isotopic enrichment of decomposing plant tissues: (1) leaching of $^{15}N$-depleted compounds, (2) C mineralization and release of $^{13}C$-depleted $CO_2$, and (3) microbial N assimilation and dissimilation, during which $^{15}N$-depleted $NH_3$ is released into soil and $^{15}N$-enriched $NH_4^+$ is left in microbial biomass (Dijkstra et al., 2008).**

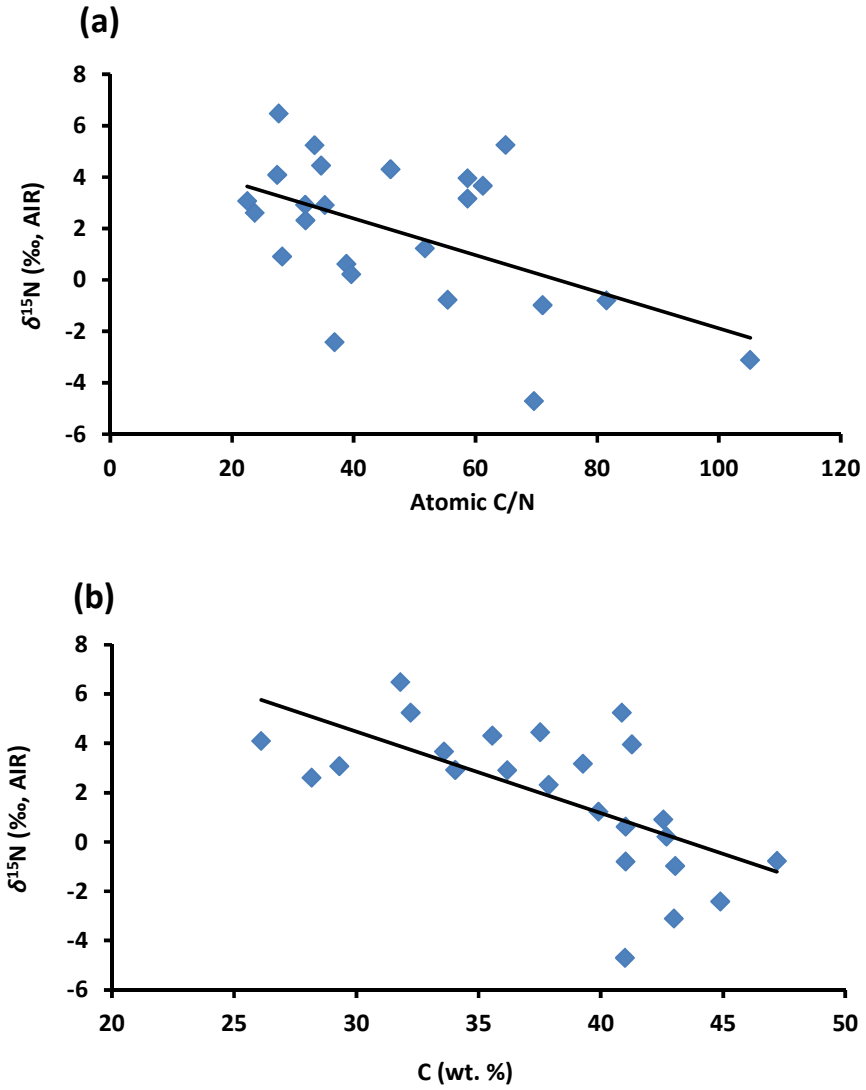


**Figure 3-19: Bivariate plot of (a) $\delta^{15}$N *vs.* atomic C/N, and (b) $\delta^{15}$N *vs.* C (wt. %) of decomposed plant tissues.**

Overall, based on the pattern of change in atomic C/N, which can be explained mostly by C loss over the course of this experiment, it is unlikely that a significant change in the N content of the decomposed plant tissues occurred. The lower average value and narrower range of atomic C/N for the 'buried' plant debris (Fig. 3-14a) likely points to a gradual increase in the contribution of microbial substrate and a shift in atomic C/N to that of the microbial products (Wallander et al., 2003). A few patterns, nonetheless, remain unexplained, for example, the steady decrease in $\delta^{15}$N of 'unburied' *P. glauca*. Despite some large fluctuations during the middle of experiment, the final atomic C/N of

'unburied' *P. glauca* did not change significantly from its initial value. It may be that the experiment had not reached a steady state condition at the end of 317 days.

The larger observation is that plant materials stored by ground squirrels in their nests are vulnerable to changes in their original N isotopic compositions. Decomposition of plant tissues at the ground surface prior to collection, however, likely had little effect on the tissue isotopic composition.

### 3.5.2 Paleosols

The paleosols analyzed here are dominated by silt, which is in agreement with previous studies of Klondike paleosols (Fraser and Burn, 1997; Sanborn et al., 2006). The clay content (avg. 19 wt. %) is higher than previously reported by Sanborn et al. 2006 (10.8 %) and Fraser and Burn 1997 (5.7 %), which suggests significant heterogeneity across the Klondike and/or systematic biases among the grain size methodologies used. The OM content (5-20 wt. %) and high abundance of fine and very fine roots in all paleosol samples suggest a base-rich environment for soil formation during the late Pleistocene and that below-ground plant parts were a major source of SOM (Sanborn et al. 2006). The paleosol mineralogy (quartz, feldspar and calcite) is similar to that of Quaternary loess elsewhere in the world (Muhs III, 2003). As has been suggested by Sanborn et al. (2006), the presence of ground squirrel nests (Fig. 3-4) and the lack of peat layers (OC > 17 wt. % (Soil Classification Working Group, 1998)) indicates well-drained conditions in the past. The average paleosol $\delta^{13}C$ of –25.6 ‰ is typical of pre-Industrial Revolution $C_3$ vegetation. The $\delta^{15}N$ of all paleosol samples vary within a narrow range (+3.4 to +4.8 ‰) except for QC-4 (+1.3 ‰). That sample, taken from a dark layer between two lighter layers (Fig. 3-6), also has the highest OM (20.4 wt. %) and OC (9.5 wt. %) contents. Ice from the nest in this layer also has most negative $\delta^{18}O$ and $\delta^{2}H$ analyzed in this study (Table 3-6). All of these features could point to its formation during a very cold period during which decomposition was limited.

### 3.5.3 Plant Macrofossils

The $\delta^{13}C$ of plant macrofossils (–27.6 to –24.7 ‰) are indicative of $C_3$ vegetation and are consistent with the ranges reported for modern plants in Chapter 2 (after correction for Suess effect), and previously reported results for both modern and late Pleistocene fossil plants from eastern Beringia (Gaglioti et al., 2011; Wooller et al., 2007, 2011). The $\delta^{15}N$ of all fossil plants are positive with the majority > +2 ‰, which is higher than that of modern plants from the region (Chapter 2) and previously reported data for both modern plants (Michelsen et al., 1996; Nadelhoffer et al., 1996; Schulze et al., 1994) and late Pleistocene macrofossils (which ranged from –4.9 to +0.5 ‰ (avg. –2.8 ‰); Wooller et al., 2011) from Arctic and subarctic regions.

The N contents of the plant macrofossils are in the range reported for modern subarctic plants in this study (Chapter 2) and those from other studies (Michelsen et al., 1996; Wang and Wooller, 2006; Wooller et al., 2007). The C contents, however, are lower than those reported for modern plants (Chapter 2 and Wooller et al., 2007), but similar to those reported by Wooller et al. (2007) for the late Pleistocene fossil plants from eastern Beringia. These results point to possible changes in the original isotopic and elemental compositions of the fossil plants, as is discussed farther in section 3.5.6.

### 3.5.4 Fossil and Modern Bone Collagen and Bioapatite Structural Carbonate

The average $\delta^{13}C_{Col}$ of fossil bones (–21.1 ± 0.8 ‰) in this study are in agreement with those reported by Bocherens et al. (2011) for four ground squirrels (–21.0 to –20.3 ‰) from Germany, which date to the post-LGM (14-12k $^{14}C$ a BP). Assuming $\Delta^{13}C_{Col\text{-}diet}$ of +3 to +4 ‰ (DeNiro and Epstein, 1978) and $\Delta^{13}C_{Sc\text{-}diet}$ of +9.9 ‰ (Ambrose and Norr, 1993; Grimes et al., 2004) for small mammals (rodents), the average $\delta^{13}C_{Col}$ (–21.1 ± 0.8 ‰) and $\delta^{13}C_{Sc}$ (–13.5 ± 1.0 ‰) of these fossil bones suggest an entirely $C_3$ diet for these animals (after correction for the Suess effect) (Cerling et al., 1997). This observation is in agreement with the results for fossil plants in this study and the paleovegetation reconstruction of Beringia reported earlier by Wooller et al. (2007, 2011). The average

$\Delta^{13}C_{Sc\text{-}Col}$ (+7.8 ± 0.6 ‰) also suggests herbivory status for these animals (Crowley et al., 2010; Lee-Thorp et al., 1989).

Two samples (GZ-3-1, GZ-3-2) from Glacier Creek (Fig. 3-2) have distinctive higher $\delta^{13}C_{Col}$ among fossil bones (~1.8 ‰), which is accompanied by slightly higher $\delta^{15}N_{Col}$ (~1 ‰). This could reflect a different diet and/or environmental conditions during life relative to the other samples. These samples date to 16,580 $^{14}$C a BP, and hence lie within the LGM, which peaked at 18k and ended at 15k $^{14}$C a BP (Fox-Dobbs et al., 2008; Clark et al., 2009; Mix et al., 2001). Eastern Beringia experienced relatively drier and colder conditions with the onset of LGM (Anderson and Brubaker, 1994; Elias, 2000), and hence higher $\delta^{13}C$ and $\delta^{15}N$ of plants and animals are expected during this period (Murphy and Bowman, 2009; Swap et al., 2004). Higher $\delta^{13}C$ has also been reported for plants from more arid environments within eastern Beringia (Wooller et al., 2007). The significantly higher $\delta^{13}C$ of the two samples from this site may also suggest some specific environmental conditions, which lead to plants with higher water use efficiency (Ehleringer and Cooper, 1988; Wooller et al., 2007). Having more samples from this site and time period could help more to clarify the main factor causing such observation.

The $\delta^{15}N_{Col}$ of fossil bones (+3.9 to +5.6 ‰) is much higher than reported by Bocherens et al. (2011) for four post-LGM ground squirrels (+1.7 to +2.5 ‰). To fully interpret these data, including any estimation of trophic level, we must first determine the N isotopic baseline of the food web for late Pleistocene Beringia. The range of $\Delta^{13}C_{Sc\text{-}Col}$ for these fossil samples (+6.9 to +8.7 ‰), however, is close to the mean reported for herbivores (~+7 ‰; Lee-Thorp et al., 1989).

The $\delta^{18}O_{dw}$ calculated for the fossil bones (QC-4, IC-9-1, IC-9-2 and IC-14) from the structural carbonate oxygen isotope data (Table 3-9) corresponds well with the $\delta^{18}O$ of ice collected from their associated nests (Table 3-6): nest ice $\delta^{18}O$ –30.7 ‰ *vs.* calculated $\delta^{18}O_{dw}$ –32.0 ‰ for QC-4, nest ice $\delta^{18}O$ –27.9 ‰ *vs.* calculated $\delta^{18}O_{dw}$ –27.0 ‰ for IC-9-1 and IC-9-2, and nest ice $\delta^{18}O$ –29.7 ‰ *vs.* calculated $\delta^{18}O_{dw}$ –29.8 ‰ for IC-14. This suggests that the structural carbonate oxygen isotopic composition provides a good representation of drinking water during the lives of these animals.

The average $\delta^{13}C_{Col}$ of modern bones compares well with that of fossil bones after correction for Suess effect. Given a $\Delta^{13}C_{Col\text{-}diet}$ of +3 to +4 ‰, the average $\delta^{13}C_{Col}$ of modern bones (–23.4 ± 0.8) also suggests an entirely $C_3$ diet for these animals (Cerling et al., 1997). The average $\delta^{13}C_{Sc}$ for these samples, however, differs between localities. At Whitehorse, the average $\delta^{13}C_{Sc}$ (–18.0 ± 0.6 ‰) indicates an entirely $C_3$ diet with overall $\delta^{13}C$ of –27.9 ‰ (assuming $\Delta^{13}C_{Sc\text{-}diet}$ = 9.9 ‰), and the average $\Delta^{13}C_{Sc\text{-}Col}$ (+5.6 ± 0.4 ‰) suggests omnivory status for these animals (Crowley et al., 2010; Lee-Thorp et al., 1989). The average $\delta^{13}C_{Sc}$ (–9.2 ± 0.5 ‰) at Kluane Lake suggests a $\delta^{13}C$ of –19.1 ‰ for their diet, which seems to imply a mixed diet of $C_4$ or $^{13}C$-rich macrophytes and more typical $C_3$ plant diet for these animals. The $\Delta^{13}C_{Sc\text{-}Col}$ (+12.7 ± 1.1 ‰) is most consistent with herbivory status for these animals.

A mixed diet of $C_3$ and $C_4$/$^{13}C$-rich macrophytes could explain the Kluane Lake carbon isotopic data for these animals if dietary protein came mainly from $C_3$ portion (i.e. $\delta^{13}C_{Col}$) and carbohydrates and lipids came from the $C_4$/$^{13}C$-rich macrophytes portion (i.e. $\delta^{13}C_{Sc}$) of the diet. Such high $\Delta^{13}C_{Sc\text{-}Col}$ is known for captive rats raised on a mixed diet of $C_3$-proteins and $C_4$-carbohydrates and lipids (Ambrose and Norr, 1993; Jim et al., 2004). A significant presence of $C_4$ plants in subarctic regions is unexpected (Sage et al., 1998; Welsh, 1974), although Wooller et al. (2007) have reported a few $C_4$ grasses from Alaska and Yukon with $\delta^{13}C$ ranging from –14 to –12 ‰. No $C_4$ plants were among our samples from Kluane Lake, but $C_4$ plants (e.g. *Muhlenbergia richardsonis*) are known from this area (Bruce Bennett Herbarium). The presence of high-$^{13}C$ macrophytes is also possible given the site's proximity to Kluane Lake. Consumption of $C_4$/high-$^{13}C$ macrophytes, which likely have low protein content (as reported for *M. richardsonis* by Dittberner and Olsen (1983)), could increase the consumer's $\delta^{13}C_{Sc}$, without significantly changing their $\delta^{13}C_{Col}$, which would mostly reflect dietary protein from $C_3$ plants.

The average $\delta^{18}O_{dw}$ calculated for the Kluane Lake bones (–19.2 ± 3.0 ‰), which represents the average $\delta^{18}O_{dw}$ of at least one year before death, is similar to that measured for Christmas Creek water from this area in late September 2012 (–22.5 ‰). The late fall precipitation that feeds Christmas Creek can be expected to have lower $\delta^{18}O$ than average

annual precipitation in this region. The average $\delta^{18}O_{dw}$ calculated for the Whitehorse structural carbonate samples (–3.7 ± 2.7 ‰), however, is very different from the $\delta^{18}O$ of nearby Schwatka Lake (–19.4 ‰). This very large difference remains unexplained at the present time.

### 3.5.5 Late Pleistocene Beringia and Possible Modern Comparisons

A modern region that mimics eastern Beringian climate, soil and vegetation compositions is prerequisite to any discussion of a possible shift in its C and N isotopic baselines between Pleistocene and present time. Previous studies note that Beringia was an ecosystem that has no exact modern analogue (Gill et al., 2009; Guthrie, 1982; Höfle et al., 2000; Williams et al., 2001). Some small portions of west central Yukon (e.g. the eastern shoreline of Kluane Lake), nonetheless, may be more or less comparable to the late Pleistocene eastern Beringia. These areas show some similarities in climatic conditions (windy, arid, low temperature), soil (continuous loess deposition, high pH, high OM content) and vegetation (an *Artemisia-Festuca* grassland) (Laxton et al., 1996). For example, paleosols from Klondike goldfields (this chapter) and modern soils from Kluane Lake (Chapter 2) have broadly similar average pH (7.6 *vs.* 7.8), and silt (71 *vs.* 50 wt. %) and OM (8.4 *vs.* 5.6 wt. %) contents, and eolian processes have been significant in their formation. It has been suggested that these soils simulating late Pleistocene Beringian soils, Laxton et al. (1996) have noted that, except for temperature, the Kluane Lake soils provide a good analogue for late Pleistocene, eastern Beringian soils.

The $\delta^{13}C_{OC}$ of the Klondike paleosols (–25.6 ± 0.3 ‰) and modern Kluane Lake soils (–24.2 ± 0.8 ‰) points to the dominance of $C_3$ plants in both ecosystems. The modern plant compositions in the Kluane Lake grasslands are similar to those reconstructed for eastern Beringia from macrofossil studies (e.g. Zazula et al., 2006b, 2007; Fraser and Burn, 1997); both are dominated by grasses (*P. glauca*, *E. trachycaulus*, *C. purpurascens,* and *L. ramosissimum*), forbs (*A. frigida*, *P. canescens*) and sedges (*C. filifolia*). Of course, local factors including elevation, topography, strength of loess deposition, drainage, aspect, slope and water content, which are superimposed upon the

regional patterns, help to determine the ecological mosaics present at both modern Kluane Lake and ancient eastern Beringia (Laxton et al., 1996; Zazula et al., 2003). Of note, the south central Whitehorse valley, located farther to the east from Kluane Lake, also has the same continental cold and dry climate and similar grassland vegetation, and thus can serve as another approximate modern equivalent to late Pleistocene eastern Beringia.

### 3.5.6 Modern and Ancient C and N Isotopic Baselines

An important assumption in interpreting the data from this study is that the ancient animals analyzed fed on plant materials similar to those recovered from their nests. The higher average $\delta^{13}C$ and $\delta^{15}N$ of fossil plants and bone collagen relative to their modern equivalents (Fig. 3-20) could point to a change in N and C isotopic food web baselines in this ecosystem between the late Pleistocene and present time. The lower average $\delta^{13}C$ of modern plants (~1.4 ‰) and modern squirrel bone collagen (~2.1 ‰) relative to their ancient counterparts can be explained by the decrease in $\delta^{13}C_{atm}$ resulting from anthropogenic activities (Suess effect: Keeling, 1979; Verburg, 2006). The $\delta^{13}C_{atm}$ was –6.4 ‰ during the LGM but has decreased steadily since the onset of the Industrial Revolution to –8.6 ‰ in 2014 (Keeling et al., 2014).

While a shift of this magnitude is present in bone $\delta^{13}C_{Col}$, a slightly smaller but likely significant difference (~1.4 ‰) is observed for plant $\delta^{13}C$. A main assumption of this study is that any change in $\delta^{13}C_{atm}$ should be reflected first in plants and then in plant consumers as it is passed through trophic levels. This discrepancy therefore suggests that there may be additional factors affecting the $\delta^{13}C$ of fossil plants.

One possibility is that microbially mediated decay of fossil plants over the time caused changes in their $\delta^{13}C$, as discussed in section 3.5.1. Such changes, however, were not apparent in the decomposition experiment. That experiment was also not able to simulate the frozen conditions in which the fossil plants were buried for thousands of years. Therefore, a possible decrease in fossil plant $\delta^{13}C$ during decomposition, which would have offset the Suess effect, cannot be ruled out.

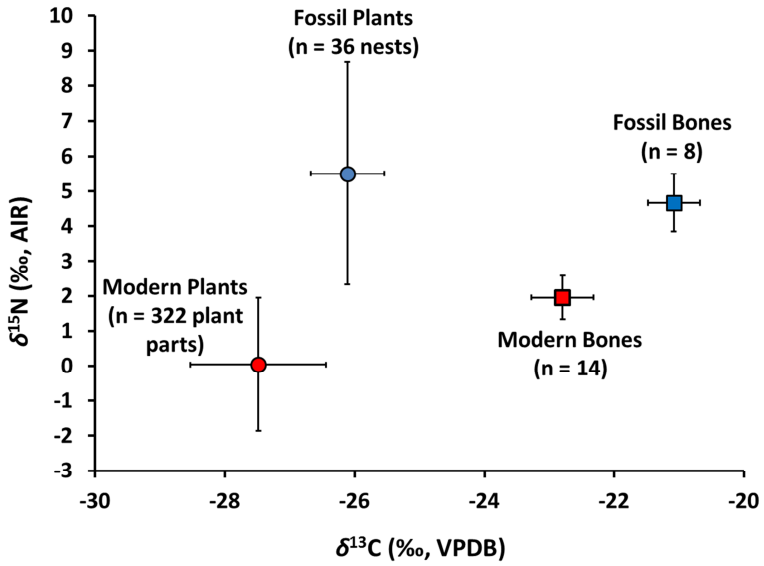


**Figure 3-20: Average $\delta^{15}$N *vs.* $\delta^{13}$C of fossil and modern samples.**

The fossil plants and fossil bone collagen also have higher average $\delta^{15}$N than their modern equivalents (Fig. 3-20). The size of this shift for plants (~5.5 ‰), however, is twice as large as it is for bones (+2.6 ‰). Part of the higher plant $\delta^{15}$N could be related to a more open N cycle in Beringia during the late Pleistocene than at present time. The discrepancy in the size of this shift between plant and bone tissues, however, suggests that other factors also may have contributed to a change in the original $\delta^{15}$N of the fossil plants. In particular, microbially mediated decay before and after burial could be a possible factor changing the original $\delta^{15}$N of the fossil plants.

There are three sets of evidence supporting the contribution of microbially mediated decay to changing the original isotopic composition of fossil plants. First, the fossil plants have higher nitrogen contents, lower carbon contents, and significantly lower atomic C/N than the modern plants (Fig. 3-21), consistent with the results of the decomposition experiment (Fig. 3-14a) and many previous studies (e.g. Connina et al., 2001; Tremblay and Benner, 2006). Second, SEM images of the surface of plant macrofossils show signs of plant tissue alteration and establishment of microbial colonies (fungal hypha and bacteria) (Fig. 3-22). Third, assuming that ground squirrels/lemmings fed on plant

materials similar to those stored in their nests, then their bone collagen should have higher $\delta^{13}C$ and $\delta^{15}N$ than the bulk fossil plants.

Not all fossil plants, however, have $\delta^{13}C$ (Fig. 3-23) and $\delta^{15}N$ (Fig. 3-24) that match those predicted from fossil bone $\delta^{13}C$ and $\delta^{15}N$ after applying $\Delta^{13}C_{\text{Col-bulk plant}}$ = +4.7 ‰ and $\Delta^{15}N_{\text{Col-bulk plant}}$ = +2.0 ‰, as calculated for modern arctic ground squirrels (Fig. 3-11). For $\delta^{13}C$, the match is good, except for one sample (GZ-3) that is depleted of $^{13}C$ by 1.1 ‰ from the estimated value (Fig. 3-23). This could support the idea that microbially mediated decay probably has caused $^{13}C$-depletion in this sample. For $\delta^{15}N$, only two nests (IC-9 and QC-4) have measured $\delta^{15}N$ close to the estimated values; plants from most nests have measured $\delta^{15}N$ that is much higher (Fig. 3-24). This difference is consistent with $^{15}N$-enrichment of the fossil plants as a product of microbially mediated decay.

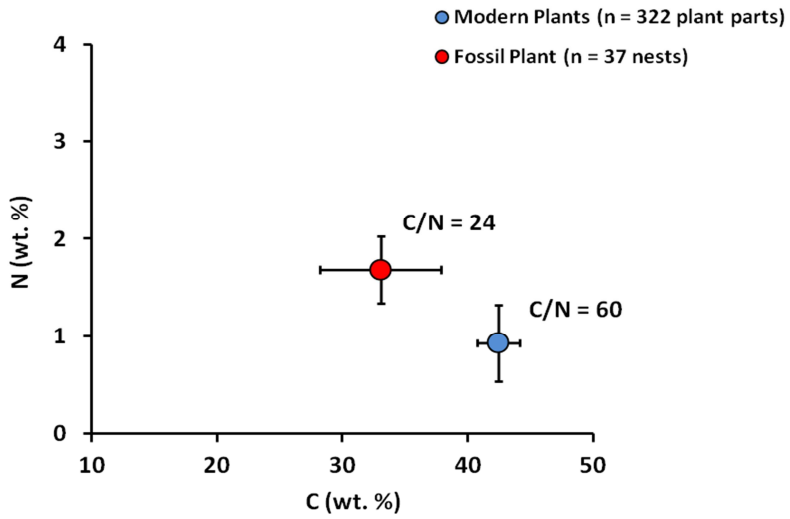


**Figure 3-21: Average N *vs.* C contents of fossil and modern plants.**

Correction of measured plant $\delta^{15}N$ and $\delta^{13}C$ to obtain original values prior to decomposition is therefore a necessary step in defining the N and C isotopic food web baselines for late Pleistocene eastern Beringia. The original plant macrofossil $\delta^{15}N$ and $\delta^{13}C$ for nests contain fossil bones, as calculated using the approach described above, is illustrated in Figure 3-25.

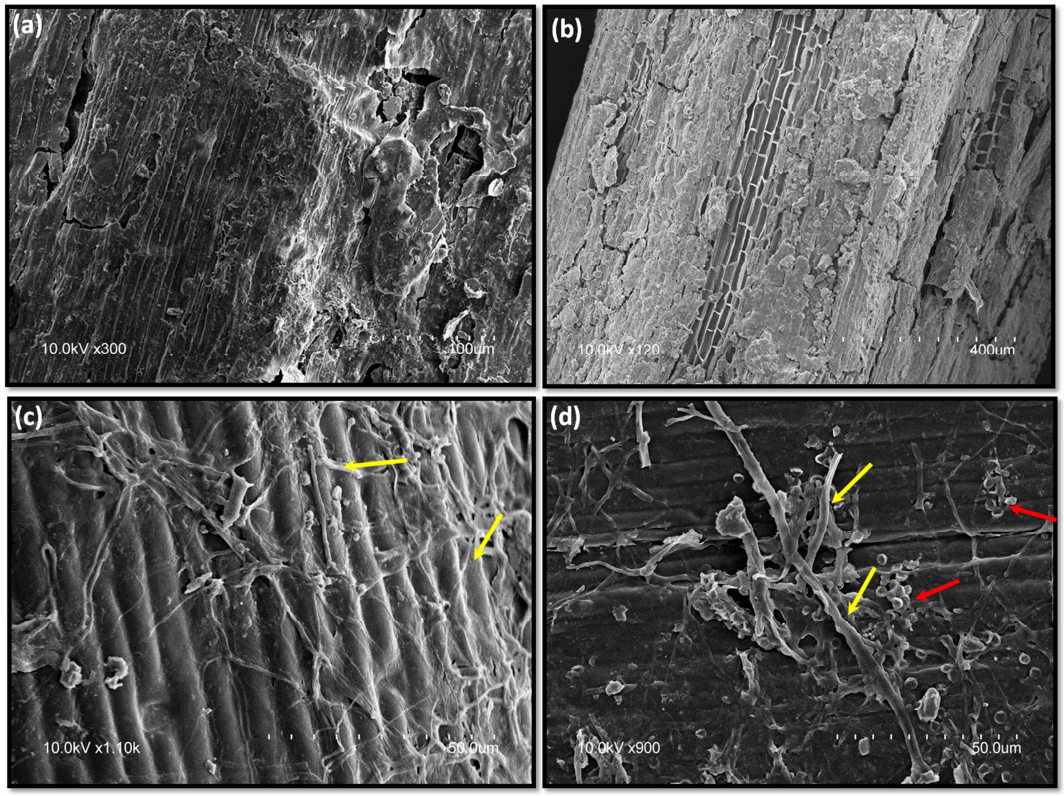


**Figure 3-22: SEM images of plant macrofossils: (a, b) decomposed surface, and (c, d) fungal hypha (yellow arrows) and bacteria cells (red arrows).**

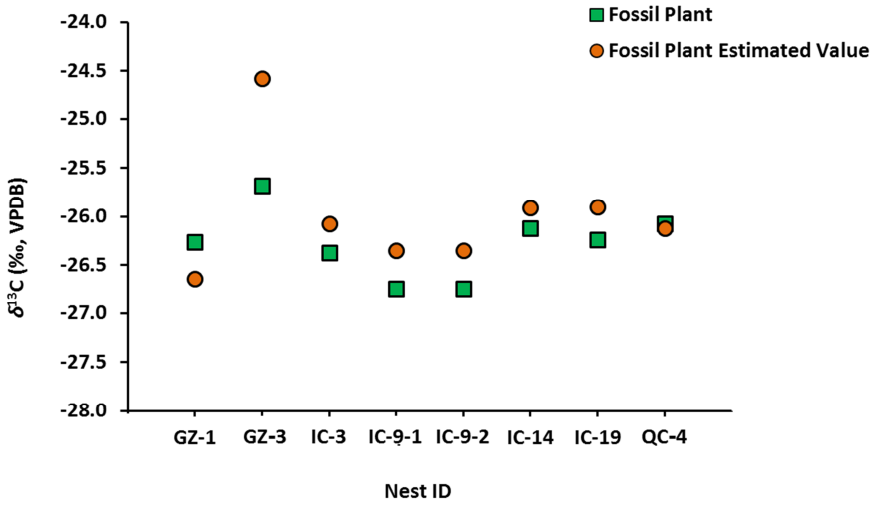


**Figure 3-23: Measured $\delta^{13}C$ *vs.* estimated $\delta^{13}C$ for fossil plants using fossil bone $\delta^{13}C$ and modern $\Delta^{13}C_{Col\text{-}bulk\ plant}$ = +4.7 ‰.**

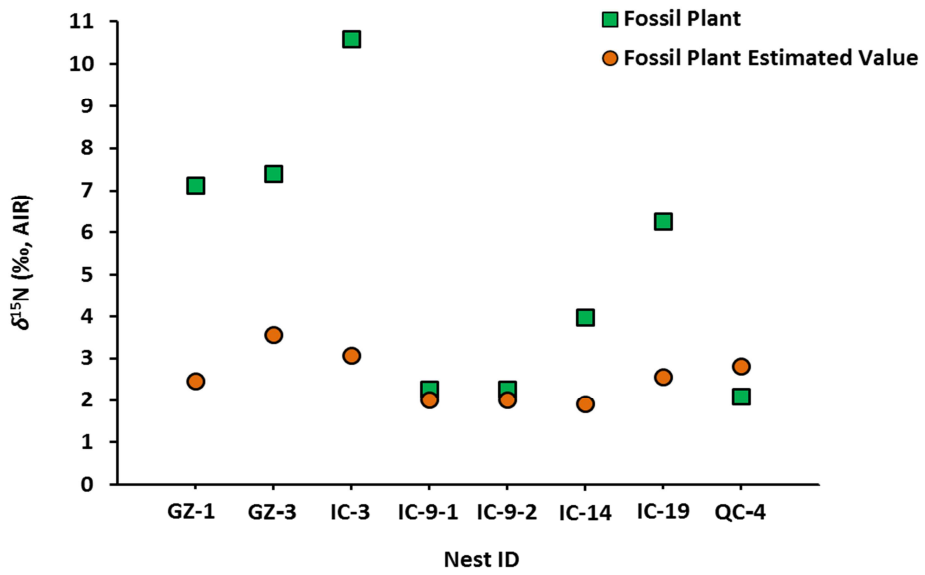


**Figure 3-24: Measured $\delta^{15}N$ *vs.* estimated $\delta^{15}N$ for fossil plants using fossil bone $\delta^{15}N$ and modern $\Delta^{15}N_{Col\text{-}bulk\ plant}$ = +2.0 ‰.**

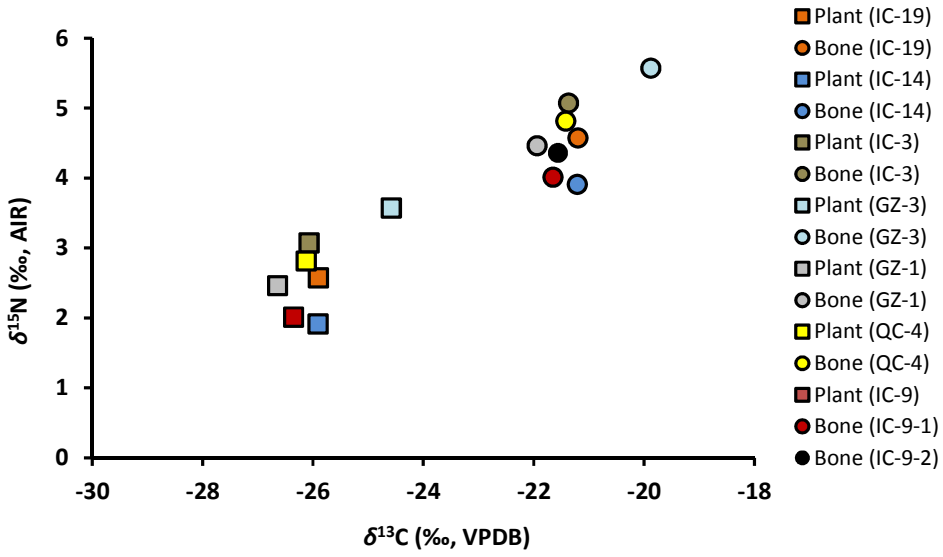


**Figure 3-25: $\delta^{15}N$ *vs.* $\delta^{13}C$ of plant macrofossils after correction for decomposition effect (Squares). The isotopic results for fossil bone collagen are also shown (Circles). Samples from the same nest are shown in the same color.**

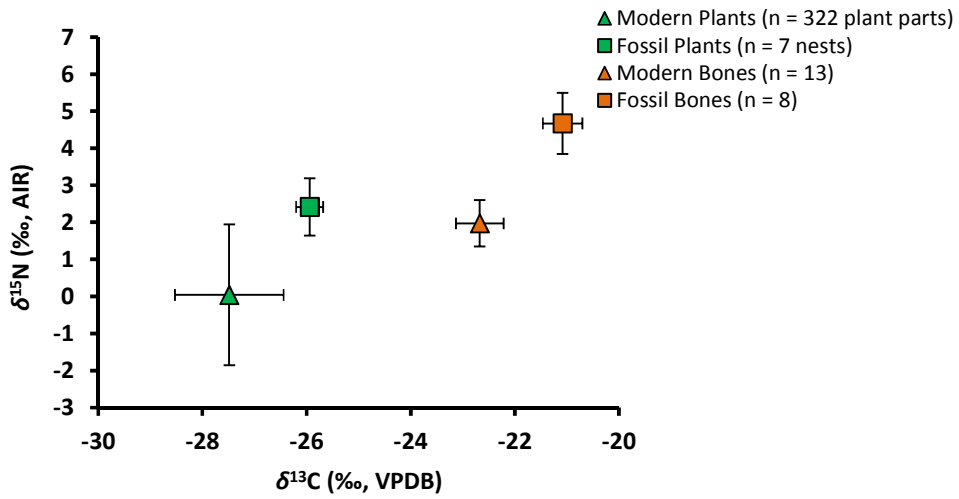


**Figure 3-26: $\delta^{15}$N *vs.* $\delta^{13}$C of fossil and modern plants and bone collagen (average $\delta^{15}$N and $\delta^{13}$C for fossil plants are calculated).**

The calculated average $\delta^{13}$C of the fossil plants is not significantly different (on average 0.2 ‰ less negative) from the measured average $\delta^{13}$C and still is higher than that of the modern plants (~1.6 ‰), which can be mainly explained by the Suess effect. The calculated, original, average $\delta^{15}$N of the fossil plants is ~2.5 ‰ higher than that of the modern plants (Fig. 3-26). This suggests greater openness of the late Pleistocene N cycle and hence a higher N isotopic composition for the food web baseline in this ecosystem at that time than at present.

Paleoecological and modern studies of arctic ground squirrels note their great selectivity when foraging and gathering plants (Zazula et al., 2006a). Nonetheless, the wide variety of plant species present in their fossil nests and the high abundance of these nests in the late Pleistocene sediments (Guthrie, 1990; Zazula et al., 2007) point to their widespread distribution in Beringia and their potential as good representation of late Pleistocene grassland vegetation, and the environmental conditions at that time.

The significant environmental changes that accompanied disappearance of large mammals at the terminal Pleistocene, together with rising atmospheric $pCO_2$ that followed could cause a shift in the nature of N cycling in eastern Beringia between then and now. Loss of the late Pleistocene large population of megafauna may be a key factor

in this change (Mann et al., 2013). Many studies suggest a driving role for herbivores in increasing N content and accelerating N cycling in ecosystems (Frank and Evans, 1997; Frank et al., 2000; McNaughton et al., 1988; Molvar et al., 1993; Pastor et al., 2006; Ruess and McNaughton, 1987). Megaherbivores can affect N cycling in ecosystems directly, by changing the rate of internal N transformations (Coetsee et al., 2010; Frank and Evans, 1997; Frank et al., 2000; Wolf et al., 2010) and the quality and amount of N input (Fox-Dobbs et al., 2012), and indirectly, by changing vegetation composition, structure and diversity (Augustine and Frank, 2001; Augustine and McNaughto, 1998; McKendrick et al., 1980; Semmartin et al., 2004; Wal et al., 2004). In ecosystems with significant herbivory, large proportions of nutrients are returned to the soil through dung and urine deposition (Haynes and Williams, 1993). This increases the N content of the ecosystem in a form that is more readily available to plants (Augustine and Frank, 2001; Augustine and McNaughton, 2006; Frank et al., 2000; Li et al., 2010; McKendrick et al., 1980; McNaughton et al., 1988; Wal et al., 2004). Long-term herbivory can also shift the composition of ecosystems toward greater abundances of more palatable species with higher N contents (Augustine and McNaughto, 1998; Coetsee et al., 2010; McKendrick et al., 1980).

The increase in $pCO_2$ that began after the terminal Pleistocene could also have changed the N content and $\delta^{15}N$ of Beringian ecosystems. Although it is not always experimentally evident (Ainsworth and Long, 2004; Langley et al., 2009), many studies support the idea that $CO_2$ enrichment above ambient levels can cause reduction in plant N contents and plant N availability (Bloom et al., 2010; Couture et al., 2012; Dyckmans et al., 1999; Garten et al., 2011; Gill et al., 2002; Liu et al., 2007; McGuire and Melillo, 1995; Peñuelas and Matamala, 1990; Reich et al., 2006a; Reich et al., 2006b; Vivin et al., 1996). It has been suggested that the increase in N sequestration into organic forms, mainly resulting from stimulated microbial N immobilization (Luo et al., 2004), and a subsequent decrease in nitrification and denitrification rates, could cause such reduction (McLauchlan et al., 2010). Other mechanisms may also be involved, including greater reliance of plants on mycorrhizal associations (McLauchlan et al., 2010), diminished nitrate assimilation as a part of the plant's acclimation to increasing $CO_2$ levels (Bloom et

al., 2010), and/or greater production of non-structural carbohydrates in plants (Korner et al., 1997). Lower plant $\delta^{15}N$ and lower plant-available N in response to $CO_2$ enrichment has been reported by Garten et al. (2011), a subject that will be examined in Chapter 4.

In a final note, while plant tissues and bone collagen of the late Pleistocene samples have higher $\delta^{15}N$ than their modern equivalents, a similar pattern in $\delta^{15}N$ is not observed for the paleosols. This may indicate that the $\delta^{15}N$ of bulk soil is not a good representation of bioavailable N in the soil (Hogberg, 1997). In addition, the depth of the paleosol samples within the original soil profile, which can affect its $\delta^{15}N$ (Hogberg, 1997; Koba et al., 1998; Makarov et al., 2008; Martinelli et al., 1999), is not known for these samples.

## 3.6 Conclusion

The nitrogen and carbon isotopic composition of plants at the base of food web in terrestrial ecosystems can be used to detect environmental change through time, including change in N cycling. Recognition of such shifts is important when undertaking paleodietary and paleoecological studies that encompass large spans of time and large landscapes.

Comparison of the C and N isotopic compositions of fossil plants and bones recovered from late Pleistocene, eastern Beringia ground squirrel nests, on one hand, with those of modern equivalents from Kluane Lake and southwest Whitehorse, Yukon, on the other hand, indicate a change in the C and N isotope food web baselines over this time. The shift in plant $\delta^{13}C$ can be explained for the most part by the Suess effect, although contribution from microbially mediated decay of fossil plant tissues cannot be ruled out. Significantly higher mean $\delta^{15}N$ of plant macrofossils relative to their modern equivalents reflect two main processes: (i) microbially mediated decay of fossil plants, and (ii) a decrease in the openness of the N cycling from the late Pleistocene in eastern Beringia to the present time at the same locality. Megaherbivores likely played an important role in eastern Beringia in facilitating a more open N cycling during the late Pleistocene. Their loss led to a gradual decrease in N content and the openness of the N cycle in this

ecosystem, a change that may have been farther amplified by the increase in atmospheric $pCO_2$.

## 3.7 References

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# Chapter 4

# 4 Response of Subarctic Terrestrial Carbon and Nitrogen Isotopic Baselines to $CO_2$ Enrichment: Controlled Experiments

## 4.1 Introduction

Assessing the impact of changing atmospheric $CO_2$ concentration ($pCO_2$) on plant nitrogen (N) and carbon (C) status is important in paleoecological studies of subarctic ecosystems. These ecosystems have experienced several glacial-interglacial cycles coincident with major global changes in atmospheric $pCO_2$ over the Quaternary Period. During the Last Glacial Maximum (LGM) (peaking at 18k $^{14}$C a BP (Fox-Dobbs et al., 2008)), terrestrial vegetation experienced one of the lowest concentrations of atmospheric $pCO_2$ (180-200 ppm) in its evolutionary history (Berner, 2006) before $pCO_2$ then increased to 270 ppm at the terminal Pleistocene to Holocene transition (12-10k $^{14}$C a BP) (Anderson and Lozhkin, 2015; Marino et al., 1992). Beginning with the Industrial Revolution (~200 years ago), $pCO_2$ has followed a steady increase to the present time (~399 ppm) (Keeling et al., 2014). Given that atmospheric $CO_2$ is the raw material for photosynthesis, farthering our understanding of how plants respond to changes in $pCO_2$, including modifications in their growth, development, nutrient status and forage quality, is an important part of ecological and environmental studies. This is particularly important in ecological studies if changes in atmospheric $pCO_2$ affect the C and N isotopic baselines of ecosystems over time.

Many studies have reported the impact of $CO_2$ enrichment on plant C isotopic compositions ($\delta^{13}$C) (Arneth et al., 2002; Nguyen Tu et al., 2004; Penuelas and Azcon-Bieto, 1992; Polley et al., 1993; Van de Water et al., 1994) and N isotopic compositions ($\delta^{15}$N) (Billings et al., 2002, 2004; Bloom et al., 2010; Garten et al., 2011; Hobbie et al., 2000; Johnson et al., 2000; Pérez-López et al., 2013; Williams et al., 2006). It is still not clear, however, which mechanisms drive such changes in plant isotopic compositions. Given that plants' response to varying $pCO_2$ has been suggested to be plant functional

group- and species-specific (Dray et al., 2014; Duval et al., 2011; Ehleringer and Cerling, 1995; Feng, 1998; Hungate et al., 1996; Zak et al., 2000b), investigating species-level responses of the most common and key vegetation of different ecosystems becomes very important.

The purpose of this study is to assess the C and N isotopic responses of three very common subarctic, perennial grass species (*Poa gluca, Elymus macrourus* and *Bromus pumpellianus*) to changes in $pCO_2$. These species were grown from seed in controlled growth-chambers under three different levels of $pCO_2$ (ambient, 800 and 1000 ppm) and three different organs (fine root, root crown and leaf) and rhizosphere soil (soils in direct and close contact with root system) were then analyzed for their C and N isotopic compositions. The pattern of isotopic response observed in this study should increase our ability to predict the possible influence of increasing $pCO_2$ on C and N isotopic baselines in subarctic regions. Tracking the N isotopic compositions of soil and various plant parts under different $pCO_2$ also provides the opportunity to investigate the possible shift in N cycling between soil and plants in response to $CO_2$ enrichment.

To evaluate possible differences in the isotopic responses of mature, well-established plants *vs.* seedlings, three grass species (*P. glauca, B. pumpellianus* and *Festuca altaica*) were also grown under the same three levels of $CO_2$ in controlled growth-chambers using root crowns (RC) collected from the eastern shoreline of Kluane Lake. The influence of grazing and dung fertilization on soil-plant N cycling and C and N isotopic compositions was also tested in a separate series of controlled growth experiments, for which null results were obtained. Those data are reported in Appendix I.

### 4.1.1 Plant C Content and $\delta^{13}C$ Variations with Change in $pCO_2$

Elevated atmospheric $CO_2$ concentrations typically promote photosynthesis and plant biomass production (Gill et al., 2002; Polley et al., 1993; Ziska et al., 1991). It also modifies the chemistry and quality of plant tissues through changing potassium, phosphorus and boron contents (Liu et al., 2007), decreasing N contents and increasing C/N and lignin/N ratios (Cotrufo et al., 1998; Couture et al., 2012; Lindroth, 2010; Norby

et al., 2001; Tuchman et al., 2003). Apart from its impact on plant-tissue chemistry, $pCO_2$ can also affect leaf morphology and physiology by changing specific leaf area and stomatal density (Woodward, 1993). Such physiological responses in turn affect plant evapotranspiration, water use efficiency (WUE) and $\delta^{13}C$ (Tieszen, 1991; Van de Water et al., 1994).

The controls on $\delta^{13}C$ of $C_3$ plants have been defined by Farquhar et al. (1989):

Equation 4.1 $$\delta^{13}C_{plant} = \delta^{13}C_{atm} - a - (b - a) \times (C_i/C_a)$$

where $\delta^{13}C_{atm}$ is the $\delta^{13}C$ of atmospheric $CO_2$ (–8.6 ‰ at the present time; Keeling et al., 2014), $a$ is the diffusive fractionation of $^{13}C$ between ambient and intercellular $CO_2$ (+4.4 ‰), $b$ is the net fractionation by RuBisCO carboxylation (ca. +28 ‰), and $C_i$ and $C_a$ are intercellular and ambient $pCO_2$, respectively. In short, the stable carbon isotopic composition of $C_3$ plants is a function of the C fixation pathway ($a$ and $b$) and any environmental factor affecting $\delta^{13}C_{atm}$ and $C_i/C_a$. The $C_i/C_a$ ratio is mainly controlled by the balance between photosynthetic demand for $CO_2$ and its supply through stomata, which in turn is affected by environmental factors such as water availability, relative humidity, irradiation and $pCO_2$ (Dawson et al., 2002; Tieszen, 1991; Yeh and Wang, 2001). The change in $pCO_2$ can affect plant $\delta^{13}C$ through modifying $C_i/C_a$ by adjusting either (i) $C_a$ or stomatal conductance, and/or (ii) changing the balance between photosynthetic $CO_2$ demand and its supply through stomata (Tieszen, 1991). Both $C_a$ and $\delta^{13}C_{atm}$ have experienced significant fluctuations throughout geological time (Marino et al., 1992; Petit et al., 1999; Schmitt et al., 2012). In order to avoid complications associated with overlapping effects of both $\delta^{13}C_{atm}$ and $C_i/C_a$, $C_i/C_a$ is expressed as discrimination against $^{13}C$ during photosynthesis ($\Delta^{13}C$), as defined for $C_3$ plants by Farquhar et al. (1989) and Farquhar and Von Caemmerer (1982):

Equation 4.2 $$\Delta^{13}C = \delta^{13}C_{atm} - \delta^{13}C_{plant} = a - (b - a) \times (C_i/C_a)$$

There are substantial variations in $\Delta^{13}C$ among different plant types and species (Ehleringer and Cerling, 1995), which are driven mainly by plant genetics and for some plant parts by environmental controls on stomatal conductance. A larger $\Delta^{13}C$ implies a

larger $C_i/C_a$ (Farquhar et al., 1989); in other words, the plant responds to the change in $C_a$ through changing stomatal conductance. Stomatal conductance is determined by stomatal size, density and the openness of stomata (Abrantes et al., 2014). Stomata allow plants to regulate their transpiration and $CO_2$ uptake (Franks and Beerling, 2009; Willmer and Fricker, 1996; Zeiger, 1983). A decrease in stomatal conductance with increase in $\Delta^{13}C$ in response to $CO_2$ enrichment has been reported in previous studies (Beerling and Chaloner, 1993; Peñuelas and Matamala, 1990; Polley et al., 1993; Van de Water et al., 1994; Woodward and Bazzaz, 1988; Woodward, 1993). Such a response, however, is not universal (Beerling, 1996; Beerling and Chaloner, 1992; Woodward, 1993) with some studies reporting a decrease in $\Delta^{13}C$ with $CO_2$ enrichment over the time (Arneth et al., 2002; Penuelas and Azcon-Bieto, 1992). Van de Water et al. (1994) reported a decrease in stomatal conductance and $\delta^{13}C$ with an increase in $C_i/C_a$ for fossil Limber pine (*pinus flexilis*) during the last deglaciation (15-12 ka).

Plants can utilize physiological strategies to keep $C_i/C_a$ constant during changing $pCO_2$ (Gerhart et al., 2012; Wong et al., 1979), which can include changing stomatal conductance (through changing stomatal size or density) (Van de Water et al., 1994; Woodward and Bazzaz, 1988), adjusting photosynthesis capacity (through changing concentration of photosynthetic enzymes) or a combination of both. Wong et al. (1979) have suggested that stomata guard cells and mesophyll cells work together to keep $C_i/C_a$ constant at very similar values for all $C_3$ plants. That said, it seems that the Limber pine described by Van de Water et al. (1994) was unable to do so during the glacial-interglacial transition, increasing in $C_i/C_a$ by 0.09. In contrast, the white carob tree (*Prosopis alba*) showed a decrease in $C_i/C_a$ of 0.08 between 1890 and 1990 as $pCO_2$ increased (Ehleringer and Cerling, 1995). Polley et al. (1993) reported a change in $C_i/C_a$ of +0.03, –0.03 and 0 for wheat, mustard and oats, respectively, over a $CO_2$ gradient from 150-350 $\mu$mol C $mol^{-1}$. While mustard and oats did not display a positive trend for $C_i/C_a$ with higher $pCO_2$, they showed a decreasing trend in $\delta^{13}C$, which suggests that additional physiological mechanisms can cause increasing plant $\Delta^{13}C$ with increasing $pCO_2$. Schubert and Jahren (2012) have suggested a potential mechanism related to the RuBisCO enzyme, completely independent of stomatal control, which could also explain

increasing plant $\Delta^{13}C$ with $CO_2$ enrichment. For activation of the RuBisCO enzyme for $CO_2$ fixation there is a need for full coordination of magnesium (Mg) at the center of eight active sites on RuBisCO. In addition to the $CO_2$ molecule required for fixation and sugar synthesis, excess $CO_2$ ("activator" $CO_2$) is needed to facilitate coordination of Mg within each RuBisCO molecule. Higher $pCO_2$ could provide additional "activator" $CO_2$ and so increase plant $CO_2$ fixation, $C_i/C_a$ and hence $\Delta^{13}C$.

Substantial variations exist in the physiological response and leaf isotopic discrimination against $^{13}C$ over different gradients of $pCO_2$, both among different species and for individuals within a population (short-lived *vs*. long-lived) (Ehleringer and Cerling, 1995; Van de Water et al., 1994). Plant species, plant organ, soil nutrient status, length of exposure to $CO_2$ enrichment and $pCO_2$ during developmental stages are among the factors contributing to the variation (Duval et al., 2011). Therefore, we can expect a nonlinear and wide range of isotopic responses of different plants to increasing $pCO_2$ in different ecosystems.

### 4.1.2 Plant N and $\delta^{15}N$ Variations with Change in $pCO_2$

The change in plant N status with changing atmospheric $pCO_2$ is an indirect response that lags the direct response of C. Hypotheses explaining the change in plant N status with $CO_2$ enrichment include: (i) a change in plant N availability because of a change in soil N cycling and microbial activities (Berntson and Bazzaz, 1997; Hagedorn et al., 2005) resulting from either increasing C allocation to soils (Billings et al., 2002, 2004; Garten et al., 2011; Hofmockel et al., 2011; Hungate et al., 1999; Langley et al., 2009; Williams et al., 2006), or increasing soil moisture caused by decreasing plant transpiration (Hungate et al., 1997; van Groenigen et al., 2011); (ii) a change in plant root architecture, length and biomass, which in turn changes plant N acquisition ability (Hofmockel et al., 2011; Rothstein et al., 2000; Zak et al., 2007); (iii) a change in plant $NO_3^-$ assimilation as a part of plant acclimation to $CO_2$ enrichment (Bloom et al., 2010; Neyra and Hageman, 1976), and (iv) a change in plant stomatal closure, transpiration and hence mass flow of soil solution into plants, which then could change plant N acquisition (McDonald et al., 2002).

Soil N availability is a function of a complex interplay among soil C and N, and microbes (Billings et al., 2002). An increase in soil C availability through an increase in plant C allocation to the rhizosphere and greater quantities of roots exudates under elevated $pCO_2$ (Cheng and Johnson, 1998; Zak et al., 2000b) could change N pools and fluxes in soil. No general model, however, has emerged to explain the response of soil N cycling to elevated $pCO_2$ across different ecosystems with different plant species compositions. Different studies present conflicting results concerning the effect of increased $pCO_2$ on the rate of N cycling: increases (Hungate et al., 1997; Zak et al., 1993), decreases (Berntson and Bazzaz, 1998), and no change (Zak et al., 2000a). In addition, a significant reduction in N availability to plants with increasing $pCO_2$ has been reported in laboratory and field studies of desert ecosystems (Billings et al., 2002; Gallardo and Schlesinger, 1995; Schaeffer et al., 2003). This could be a result of increases in microbial N immobilization.

A reduction in ecosystem N availability caused by long-term $CO_2$ enrichment may also be reflected in plant $\delta^{15}N$. Billing et al. (2002) reported an increase in $\delta^{15}N$ of two perennial shrubs over 7 months as $pCO_2$ was changed from ambient to 550 ppm. Williams et al. (2006) reported similar $^{15}N$ enrichment of tallgrass prairie plants and $^{15}N$ depletion of soil (5-30 cm depth) over 7 months of $CO_2$ enrichment (700-720 ppm). In contrast, Garten et al. (2011) reported a significantly faster decline in leaf litterfall $\delta^{15}N$ for sweetgum (*Liquidambar styraciflua*) trees over 11 years free-air $CO_2$ enrichment experiment (FACE) (544 ppm) and related that to a decline in soil N availability and openness of N cycling.

## 4.2 Materials and Methods

### 4.2.1 Design of Experiments and Growth Conditions

Three grass species, *P. glauca*, *E. macrourus* and *B. pumpellianus*, which were probably among the most abundant grasses in Beringia during the late Pleistocene (Swanson, 2006; Zazula et al., 2007), were germinated from seeds in small plastic pots (see below) in a greenhouse (at ~15°C, which is appropriate for cool-season grasses; Deering and Young, 2006; Palazzo and Brar, 1997). Seeds of *P. glauca* and *E. macrourus* were provided by

Randy Lewis (Arctic Alpine Reclamation group) and seeds of *B. pumpellianus* were collected in 2014 from the eastern shoreline of Kluane Lake, Yukon Territory. The 4-day-old seedlings were then transported to controlled growth-chambers (Model: GCW15 chamber, Environmental Growth-Chambers, Chagrin Falls, OH, USA) at the Biotron Centre for Experimental Climate Change Research at the University of Western Ontario, where they were maintained in separate chambers at ambient $CO_2$ (411 ± 20 $\mu$mol C $mol^{-1}$/ppm) (T0) and two elevated levels of $CO_2$ (794 ± 12 (T1) and 992 ± 10 $\mu$mol C $mol^{-1}$/ppm (T2)) for 60 days. The growth conditions of all chambers were set at the day/night temperature (T) regimes of 15/10°C (mean daily June and July T for Yukon Territory at ~63°N) (Kusterer, 2012), a photosynthetic photon flux density (PPFD) of 1000 ± 200 $\mu$mol photons $m^{-2}s^{-1}$, 50-60 % relative humidity (RH) and a 19/5 h photoperiod (PP) (mean daily June and July daylight hours in Yukon Territory at ~63°N). Root crowns of three grass species, *P. glauca*, *B. pumpellianus* and *Festuca altaica,* which were also collected from the eastern shoreline of Kluane Lake, Yukon Territory in 2014, were also grown in plastic pots in these controlled growth-chambers at three different levels of $CO_2$ at the same time and under the same conditions as the seedlings.

Each level of $CO_2$ treatment was replicated in three separate growth-chambers with three replicates for each species grown from seeds and one for each species grown from root crowns sampled from each chamber. In total, nine replicates of each species grown from seeds and three replicates of each species grown from root crowns were analyzed for each level of $pCO_2$.

The required $CO_2$ concentrations above ambient (800 and 1000 $\mu$mol C $mol^{-1}$/ppm) were supplied using cylinders containing pure $CO_2$ (Praxair, Mississauga, ON, Canada), with the amount added controlled using a pressure regulator with a constant flow rate (Praxair, Mississauga, ON, Canada). The $CO_2$ concentrations were maintained at constant levels 24 h/d for the plant growth period (60 d) and were monitored continuously using a computer-controlled, $CO_2$ infrared gas analyzer (Model: WMA-4 $CO_2$ Analyzer, PP Systems International, Inc., Amesbury, MA, USA) installed in each chamber. Other

environmental conditions (T, RH, PPFD and PP) for each chamber were also computer-controlled continuously.

The seeds were grown in free-draining (perforated at the base) square plastic pots (13 cm sides × 14 cm height; ~2.4 L). Root crowns were grown in larger, round plastic pots (15 cm radius × 37 cm height; ~26.1 L). The size of pots required to avoid unintended effects of becoming pot-bound were estimated for seedlings based on the rooting depth and lateral root spread of seedlings grown earlier for 2 months. For root crowns, the pot sizes were estimated based on the size of root systems reported for perennial grasses in water-limited ecosystems (Schenk and Jackson, 2002).

For both germination and growth steps, the plants were grown in loess soil collected from eastern shoreline of Kluane Lake, Yukon Territory, and fertilized with compost cow manure (N = 2 wt. % and $\delta^{15}N$ = +1.5 ‰) in two equal applications (60 kg N/ha: 5.1 g fertilizer/2.4 L soil and 21.5 g fertilizer/26.1 L soil) at days 1 and 17. Prior to use, the cow manure was oven-dried (90°C overnight), ground using a metal pestle and mortar and farther homogenized by passage through a 2-mm sieve. The cow manure was gently stirred into the top 10 cm of soil in order to limit N loss through volatilization. Watering was performed every two days using tap water and a watering can with a gentle flow rate. Water was added until the soil surface appeared completely wet and fully infiltrated. Over the course of the experiments, all pots were relocated randomly every 7 days within each growth-chamber to account for micro-variations in light, temperature and/or humidity.

### 4.2.2 Sample Collection

At the end of 60 days, three different plant parts (fine root, root crown and leaf) plus rhizosphere soil were sampled from all pots for C and N elemental and isotopic analyses. In addition, for each $CO_2$ treatment, five individual air samples were collected from different spots of each chamber using septum-sealed glass vials left to equilibrate for three days with the chamber atmosphere. These samples were then used to obtain the $\delta^{13}C$ of the chamber $CO_2$ ($\delta^{13}C_{CO2}$). Sampling of pure $CO_2$ from the $CO_2$ tanks supplying

the growth chambers was performed by controlled transfer to, and equilibration with a ~150 ml two port glass flask via a step-down pressure regulator.

### 4.2.3 Sample Preparation

Soil and plant materials were prepared for elemental and isotopic analyses following the procedures described for soil and plant samples in Chapter 2, section 2.2.1.

### 4.2.4 Elemental Analysis

The OC and TN contents (dry wt. %) of plant samples and TN contents (dry wt. %) of soil samples were determined using the same instruments, protocols, standards and calibrations described for soil and plant samples in Chapter 2, section 2.2.2. The average C and N contents obtained for the internal keratin standard were 48.21 ± 0.81 wt. % (n = 53) and 14.66 ± 0.25 (n = 63), respectively, which compare well with their expected values of 48.22 ± 1.07 wt. % and 14.85 ± 0.43 wt. % (n = 261). Sample reproducibility of duplicates for C was ± 0.25 wt. % (n = 29). The average N content for NIST 1547 was 2.83 ± 0.05 wt. % (n = 43), which compares well with its accepted value of 2.94 wt. %. Sample reproducibility of duplicates for N was ± 0.02 wt. % (n = 34).

### 4.2.5 Isotopic Analysis

The C and N isotopic compositions of plant C and N, and the N isotopic composition of soil TN, were measured following the same protocols described in Chapter 2, section 2.2.3. Typical sample sizes for $\delta^{13}C$ measurements were 0.4 mg for plants and for $\delta^{15}N$, ~1-3 mg for plants and ~15-30 mg for soil. To analyze air samples, septum-sealed glass vials containing growth-chamber air samples were placed in a Thermo Scientific GasBench block. The air was then automatically transferred from the GasBench device coupled to a Delta$^{plus}$ XL-CF-IRMS in dual-inlet mode for measurement of $\delta^{13}C_{CO2}$. Pure $CO_2$ sampled from tanks was analyzed for $\delta^{13}C_{CO2}$ using a VG Optima IRMS or VG Prism IRMS in dual-inlet mode. The $\delta^{13}C_{CO2}$ of growth-chamber and tank samples were calibrated to VPDB using NBS-19 (accepted value: $\delta^{13}C$ = +1.95 ‰) and L-SVEC (accepted value: $\delta^{13}C$ = –46.60 ± 0.2 ‰) (Coplen et al., 2006).

Accuracy and precision of the isotopic analyses were monitored using the laboratory keratin and IAEA-CH-6 (sucrose) standards for soil and plant samples. The average $\delta^{13}C$ obtained for keratin was –24.09 ± 0.06 ‰ (n = 52), which compares well with its accepted value of –24.05 ± 0.15 ‰. The average $\delta^{13}C$ obtained for IAEA-CH-6 was –10.51 ± 0.10 ‰ (n = 24), which compares well with its accepted value of –10.45 ± 0.03 ‰ (Coplen et al., 2006). Sample reproducibility for duplicates was ± 0.10 ‰ for $\delta^{13}C$ (n = 25). The average $\delta^{15}N$ of keratin was +6.40 ± 0.09 ‰ (n = 63), which compares well with its accepted value of +6.36 ‰. Sample reproducibility for duplicates was ± 0.09 ‰ for $\delta^{15}N$ (n = 31). Sample reproducibility for duplicate analyses of growth-chamber $\delta^{13}C_{CO2}$ was ± 0.14 ‰ (n = 9). The accuracy and precision of all standards associated with data presented in this chapter are listed in Appendix J.

### 4.2.6 Statistical Analysis

Comparisons of C and N isotopic and elemental compositions for: (i) different plant parts within each $CO_2$ treatment, and (ii) different plant parts grown under different $CO_2$ treatments were performed using one-way ANOVA followed by means comparison using either Tukey's test, if variance was homogeneous, or Dunnett's test, if variance was not homogeneous. Comparison between mean isotopic and elemental responses of *F. altaica* in experiments T0 and T1 were conducted using Independent-samples t-test. All statistical analyses were performed using SPSS 20.

## 4.3 Results

### 4.3.1 Carbon Isotopic Composition of $CO_2$ in Growth-Chambers

The $\delta^{13}C_{CO2}$ of the supply tanks for experiments T1 and T2 is –10.6 ± 0.2 ‰ (Table 4-1). The average $\delta^{13}C_{CO2}$ of each growth-chamber during each treatment is listed in Table 4-1. These samples were taken from inside the growth-chambers, which are contained within a building; hence their $\delta^{13}C_{CO2}$ could be affected by building ventilation as well as other factors. The $\delta^{13}C_{CO2}$ of all chambers for experiments T0 and T1 is almost the same, but is higher for experiment T2. Tank $\delta^{13}C_{CO2}$ used to increase the $CO_2$ concentration, however, is identical for both T1 and T2. The difference in growth-chamber $\delta^{13}C_{CO2}$ may arise in

part from seasonal changes in ambient $\delta^{13}C_{CO2}$ (Keeling et al., 2014). The T0 and T1 experiments were conducted during December 2014-January 2015 and February-March 2015, respectively, during the cold season when regional vegetation is not physiologically active and ambient $\delta^{13}C_{CO2}$ is lower. A possible larger contribution of lower $\delta^{13}C_{CO2}$ in the air from increased winter emissions from interior heating and vehicles should also not be overlooked. Experiment T2 was conducted during April-May 2015 when regional vegetation becomes active, thus causing ambient $\delta^{13}C_{CO2}$ to increase because of photosynthetic discrimination against $^{13}C$. In addition, the influence of anthropogenically produced $CO_2$ from vehicle and heating systems would be reduced. Higher $pCO_2$ in the growth-chambers during experiment T2 also probably helped to minimize the isotopic effects of ambient $CO_2$ contamination. To account for the offset in growth-chamber $\delta^{13}C_{CO2}$ between experiments T2 and T0-T1, all growth-chamber plant tissue $\delta^{13}C$ values were normalized to the value obtained for $\delta^{13}C_{CO2}$ from growth-chamber T0.

**Table 4-1: Average growth-chamber $\delta^{13}C_{CO2}$.**

| Treatment | $\delta^{13}$C-$CO_2$ (‰, VPDB) | | | |
|---|---|---|---|---|
| | Chamber 1 | Chamber 2 | Chamber 3 | Average ± SD |
| T0 | −11.5 (± 0.3)[a] | −11.1 (± 0.2) | −12.3 (± 0.1) | −11.6 (± 0.5) |
| T1 | −12.1 (± 0.3) | −11.8 (± 0.1) | −11.8 (± 0.2) | −11.9 (± 0.3) |
| T2 | −10.2 (± 0.3) | −10.2 (± 0.2) | −10.3 (± 0.2) | −10.2 (± 0.2) |
| Tank $CO_2$ | - | - | - | −10.6 (± 0.2)[b] |

**[a] Values in parentheses are SD of replicates (n = 5).**

**[b] Average of 6 $CO_2$ samples collected from tanks during T1 and T2 experiments.**

## 4.3.2 Carbon Isotopic Results

### 4.3.2.1 Intra-plant Variation in $\delta^{13}C$ within $CO_2$ Treatments

The average $\delta^{13}C$ of three different plant parts (fine root, root crown and leaf) of four different plant species (seedlings grown from seeds: *P. glauca*, *B. pumpellianus*, *E. macrourus*; plants grown from root crowns (henceforth referred to as mature plants): *P. glauca*, *B. pumpellianus*, *F. altaica*) grown under the three different $CO_2$ treatments are summarized in Table 4-2. Individual C isotopic results are provided in Appendix K. No

root crowns of *F. altaica* were available for experiment T2. For all $CO_2$ treatments and all species, the average $\delta^{13}C$ of leaves are more negative than fine roots and root crowns, except for *F. altaica* in T0 (Table 4-3). The average difference in $\delta^{13}C$ between root crown and leaf ($\Delta^{13}C_{RC-L}$) among all plants and treatments ranges from +0.2 ± 2.1 ‰ to +1.8 ± 0.3 ‰ and the average difference in $\delta^{13}C$ between fine root and leaf ($\Delta^{13}C_{FR-L}$) ranges between +0.1 ± 1.1 ‰ and +3.7 ± 0.5 ‰. These differences are not statistically significant in all cases (Table 4-4), but show a general pattern of leaves having more negative $\delta^{13}C$ than non-photosynthesizing/heterotrophic tissues (fine roots and root crowns).

**Table 4-2: Average $\delta^{13}C$ and $\delta^{15}N$ of plant parts and rhizosphere soil for different $CO_2$ treatments**

| Plant Species | Plant part | $\delta^{13}C$ (‰, VPDB) | | | $\delta^{15}N$ (‰, AIR) | | |
|---|---|---|---|---|---|---|---|
| | | T0 | T1 | T2 | T0 | T1 | T2 |
| ***E. macrourus* (S)** | **FR** | −28.5 (± 0.3)* | −28.9 (± 1.4) | −30.6 (± 1.0) | −1.4 (± 1.3) | +0.9 (± 1.2) | +2.2 (± 0.7) |
| | **RC** | −29.0 (± 1.1) | −29.8 (± 2.1) | −30.6 (± 0.7) | −0.3 (± 1.2) | −0.3 (± 2.6) | +2.9 (± 1.0) |
| | **L** | −30.3 (± 1.0) | −30.9 (± 1.9) | −31.7 (± 1.6) | +0.2 (± 1.2) | +0.5 (± 2.9) | +4.2 (± 0.5) |
| | **Soil** | - | - | - | +5.1 (± 0.2) | +4.6 (± 0.4) | +4.9 (± 0.4) |
| ***P. glauca* (S)** | **FR** | −26.1 (± 0.4) | −27.9 (± 0.5) | −29.4 (± 0.6) | −1.0 (±0.9) | +2.1 (± 1.3) | −0.5 (± 1.5) |
| | **RC** | −26.4 (± 0.4) | −28.8 (± 0.8) | −29.3 (± 0.7) | −0.8 (± 0.9) | +1.1 (± 2.1) | −0.9 (± 1.6) |
| | **L** | −28.2 (±0.5) | −30.1 (± 0.9) | −30.8 (± 1.2) | −1.6 (± 1.0) | +2.1 (± 1.7) | +0.5 (± 1.8) |
| | **Soil** | - | - | - | +4.7 (± 0.1) | +4.5 (± 0.4) | +5.0 (± 0.3) |
| ***B. pumpellianus* (S)** | **FR** | −28.6 (± 1.2) | −28.6 (± 0.8) | −29.0 (± 0.9) | −0.8 (± 0.8) | +0.7 (± 1.1) | +3.5 (± 0.8) |
| | **RC** | −28.1 (± 1.6) | −28.1 (± 1.1) | −28.6 (± 0.7) | −1.1 (± 1.1) | + 0.1 (± 1.6) | +1.7 (± 0.7) |
| | **L** | −29.8 (± 1.6) | −29.3 (± 0.9) | −29.6 (± 0.6) | +0.9 (± 1.1) | +1.4 (± 1.0) | +4.7 (± 0.6) |
| | **Soil** | - | - | - | +5.1 (± 0.2) | +4.8 (± 0.2) | +4.9 (± 0.3) |
| ***P. glauca* (RC)** | **FR** | −28.5 (± 1.6) | −29.9 (± 1.3) | −31.3 (± 1.5) | +0.8 (± 1.6) | −1.3 (± 0.5) | −1.2 (± 0.5) |
| | **RC** | −29.1 (± 1.1) | −30.6 (± 0.4) | −30.9 (± 1.1) | − 0.1 (± 2.6) | −2.4 (± 1.1) | −2.5 (± 0.7) |
| | **L** | −30.0 (± 0.8) | −31.8 (± 0.0) | −31.4 (± 1.7) | +2.3 (± 1.0) | −0.2 (± 2.0) | +1.5 (± 1.3) |
| | **Soil** | - | - | - | +2.9 (± 1.0) | +1.9 (± 0.6) | +1.5 (± 1.4) |
| ***B. pumpellianus* (RC)** | **FR** | −27.9 (± 1.0) | −28.1 (± 2.2) | −28.4 (± 1.6) | +1.1 (± 0.4) | −1.1 (± 0.6) | +0.2 (± 0.2) |
| | **RC** | −28.4 (± 1.1) | −28.2 (± 1.3) | −29.2 (± 1.1) | −0.2 (± 0.5) | −1.7 (± 1.4) | +0.3 (± 1.4) |
| | **L** | −29.5 (± 1.8) | −28.5 (± 0.0) | −29.4 (± 2.3) | +2.6 (± 1.2) | +1.2 (± 0.9) | +3.1 (± 2.0) |

**Table 4-2. Cont'd.**

| Plant Species | Plant | $\delta^{13}$C (‰, VPDB) | | | $\delta^{15}$N (‰, AIR) | | |
|---|---|---|---|---|---|---|---|
| | part | T0 | T1 | T2 | T0 | T1 | T2 |
| *B. pumpellianus* (RC) | Soil | - | - | - | +4.6 (± 0.3) | +2.7 (± 1.5) | +4.3 (± 0.6) |
| *F. altaica* (RC) | FR | −28.6 (± 1.5) | −26.7 (±0.2) | - | +1.2 (± 0.5) | −0.2 (± 0.3) | - |
| | RC | −29.0 (± 1.0) | −29.1 (± 0.4) | - | +0.2 (± 2.2) | −2.2 (± 0.2) | - |
| | L | −28.5 (± 1.6) | −30.4 (± 0.6) | - | +1.7 (± 1.1) | −0.1 (± 0.5) | - |
| | Soil | - | - | - | +4.3 (± 1.6) | +2.2 (± 1.1) | - |

**FR: fine root; RC: root crown, L: leaf.**

**T0: ambient $CO_2$; T1: $CO_2$ = 800 ppm; T2: $CO_2$ = 1000 ppm.**

**(S): grown from seed; (RC): grown from root crown.**

***Values in parentheses are SD of replicates (n = 9 for seedlings, and n = 3 for plants grown from root crowns).**

**Table 4-3: Average $\Delta^{13}C_{RC\text{-}L}$ and $\Delta^{13}C_{FR\text{-}L}$ for each $CO_2$ treatment.**

| Treatment | Plant Species | $\Delta^{13}C_{RC\text{-}L}$ | $\Delta^{13}C_{FR\text{-}L}$ |
|---|---|---|---|
| | | (‰, VPDB) | |
| T0 | *P. glauca* (S) | +1.8 (± 0.3)* | +2.1 (± 0.4) |
| | *P. glauca* (RC) | +0.9 (± 0.6) | +1.5 (± 0.7) |
| | *E. macrourus* (S) | +1.3 (± 0.3) | +1.7 (± 0.9) |
| | *B. pumpellianus* (S) | +1.7 (± 0.4) | +1.3 (± 0.7) |
| | *B. pumpellianus* (RC) | +1.1 (± 1.1) | +1.6 (± 1.1) |
| | *F. altaica* (RC) | −0.5 (± 0.8) | −0.1 (± 1.0) |
| T1 | *P. glauca* (S) | +1.2 (± 0.6) | +2.2 (± 0.8) |
| | *P. glauca* (RC) | +1.2 (± 0.4) | +1.8 (± 1.3) |
| | *E. macrourus* (S) | +1.1 (± 1.9) | +2.0 (± 1.2) |
| | *B. pumpellianus* (S) | +1.2 (± 0.3) | +0.7 (± 0.3) |
| | *B. pumpellianus* (RC) | +0.3 (± 1.3) | +0.4 (± 2.1) |
| | *F. altaica* (RC) | +1.3 (± 0.3) | +3.7 (± 0.5) |
| T2 | *P. glauca* (S) | +1.3 (± 0.8) | +1.3 (± 0.8) |
| | *P. glauca* (RC) | +0.5 (± 0.6) | +0.1 (± 1.1) |
| | *E. macrourus* (S) | +1.7 (± 0.5) | +1.2 (± 1.1) |
| | *B. pumpellianus* (S) | +1.0 (± 0.4) | +0.6 (± 0.7) |
| | *B. pumpellianus* (RC) | +0.2 (± 2.1) | +1.0 (± 1.3) |
| | *F. altaica* (RC) | - | - |

**T0: ambient $CO_2$; T1: $CO_2$ = 800 ppm; T2: $CO_2$ = 1000 ppm.**
**(S): grown from seed; (RC): grown from root crown.**
***Values in parentheses are SD of replicates (n = 9 for seedlings, and n = 3 for plants grown from root crowns).**

**Table 4-4: One-way ANOVA *p*-value results for $\Delta^{13}C$ among plant tissues for each $CO_2$ treatment.**

| Plant Species | | Treatment | | | | | |
|---|---|---|---|---|---|---|---|
| | | T0 | | T1 | | T2 | |
| | Tissue | RC | FR | RC | FR | RC | FR |
| ***P. glauca*** **(S)** | **L** | **0.000** | **0.000** | **0.033** | **0.001** | **0.009** | **0.011** |
| | **RC** | - | 0.557 | - | 0.197 | - | 0.997 |
| ***P. glauca*** **(RC)** | **L** | 0.635 | 0.348 | 0.058 | 0.257 | 0.890 | 0.993 |
| | **RC** | - | 0.839 | - | 0.764 | - | 0.934 |
| ***E. macrourus*** **(S)** | **L** | **0.047** | **0.002** | 0.434 | 0.076 | 0.153 | 0.113 |
| | **RC** | - | 0.568 | - | 0.558 | - | 0.994 |
| ***B. pumpellianus*** **(S)** | **L** | **0.049** | 0.182 | **0.030** | 0.251 | **0.036** | 0.251 |
| | **RC** | - | 0.780 | - | 0.522 | - | 0.578 |
| ***B.s pumpellianus*** **(RC)** | **L** | 0.581 | 0.362 | NA | NA | 0.994 | 0.779 |
| | **RC** | - | 0.899 | - | NA | - | 0.835 |
| ***F. altaica*** **(RC)** | **L** | 0.913 | 0.996 | **0.025** | **0.000** | NA | NA |
| | **RC** | - | 0.943 | - | **0.001** | - | NA |

**L: Leaf; RC: Root crown; FR: Fine root.**

**T0: ambient $CO_2$; T1: $CO_2$ = 800 ppm; T2: $CO_2$ = 1000 ppm.**

**(S): grown from seed; (RC): grown from root crown.**

**Values in boldface font are statistically significant ($p \leq 0.05$).**

**NA: comparison of means not conducted (lack of sample or replicates < 3).**

#### 4.3.2.2 $\delta^{13}C$ Variations among $CO_2$ Treatments

Plant C isotopic responses to the different $CO_2$ treatments are reported here in two parts: (i) seedlings, and (ii) mature plants (grown from root crowns). All plant parts of all seedlings show a decrease in $\delta^{13}C$ from T0 to T1 to T2 except for leaves of *B. pumpellianus* (Fig. 4-1). This change is statistically significant for *P. glauca* for all parts from T0 to T1 and T0 to T2, and for *E. macrourus* only for fine roots from T0 to T2, and T1 to T2 (Table 4-5). Mature plants (*P. glauca* and *B. pumpellianus*) show almost the same pattern as their seedlings (Fig. 4-2), but the difference is not significant. In general, there is no statistically significant change in $\delta^{13}C$ for different plant parts among the mature plants at elevated $CO_2$ concentrations (Table 4-5). No significant or consistent variation in $\Delta^{13}C_{\text{other plant part-leaf}}$ is observed with $CO_2$ enrichment for either seedlings or mature plants (Table 4-3).

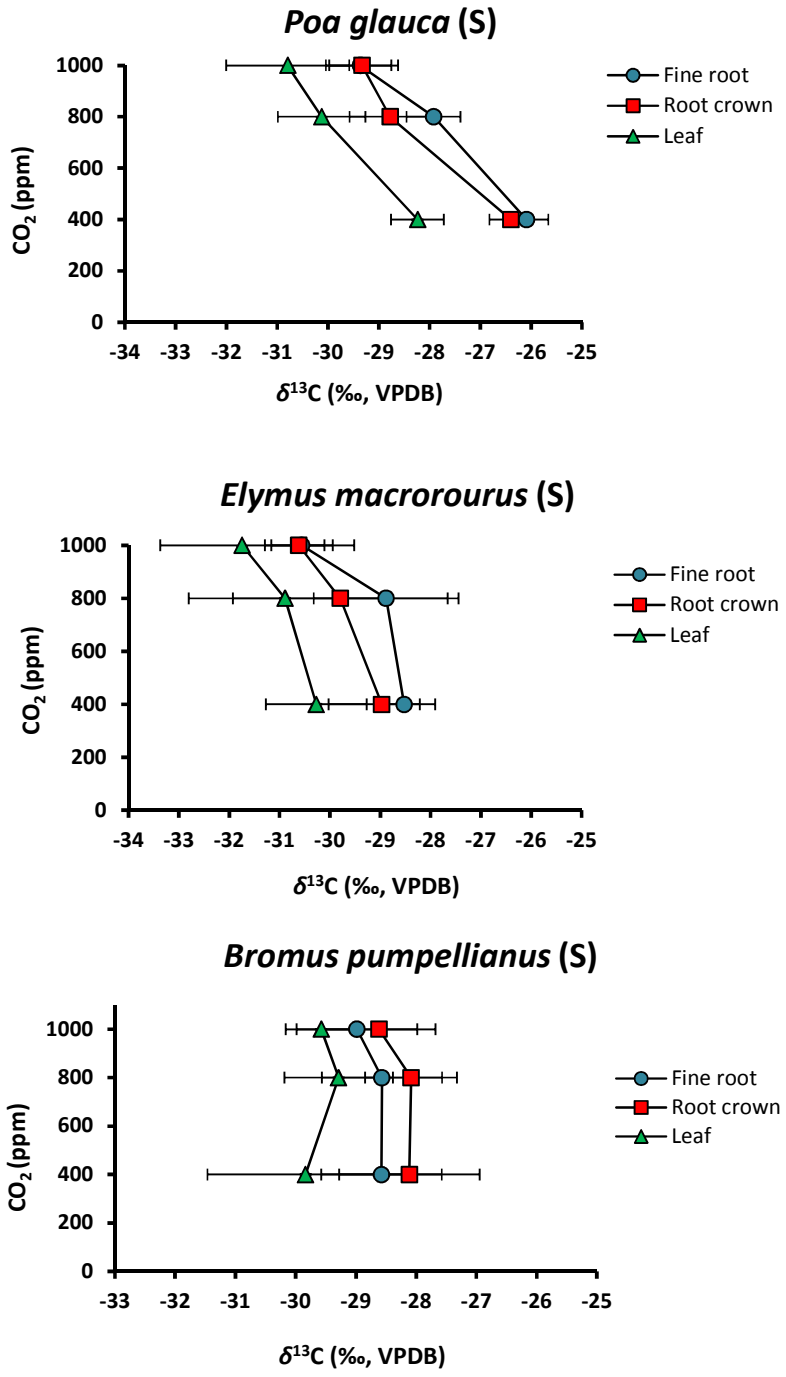


**Figure 4-1: Change in $\delta^{13}C$ of seedlings with $CO_2$ enrichment (ambient, 800 and 1000 ppm). Averages ± SD (n = 6-9) are illustrated.**

**Table 4-5: One-way ANOVA *p*-value results for differences in $\delta^{13}C$ and $\delta^{15}N$ of different plant parts between $CO_2$ treatment.**

| Plant Species | Tissue | Treatment | $\delta^{13}C$ (‰, VPDB) | | $\delta^{15}N$ (‰, AIR) | |
|---|---|---|---|---|---|---|
| | | | T1 | T2 | T1 | T2 |
| ***P. glauca* (S)** | **L** | **T0** | **0.023** | **0.001** | **0.003** | 0.095 |
| | | **T1** | - | 0.306 | - | 0.135 |
| | **RC** | **T0** | **0.000** | **0.000** | 0.159 | 0.986 |
| | | **T1** | - | 0.315 | - | 0.078 |
| | **FR** | **T0** | **0.000** | **0.000** | **0.005** | 0.839 |
| | | **T1** | - | **0.001** | - | **0.007** |
| | **Soil** | **T0** | - | - | 0.946 | 0.151 |
| | | **T1** | - | - | - | 0.065 |
| ***P. glauca* (RC)** | **L** | **T0** | 0.184 | 0.300 | 0.170 | 0.793 |
| | | **T1** | - | 0.919 | - | 0.382 |
| | **RC** | **T0** | 0.173 | 0.109 | 0.272 | 0.243 |
| | | **T1** | - | 0.931 | - | 0.995 |
| | **FR** | **T0** | 0.502 | 0.124 | 0.099 | 0.123 |
| | | **T1** | - | 0.515 | - | 0.985 |
| | **Soil** | **T0** | - | - | 0.486 | 0.306 |
| | | **T1** | - | - | - | 0.913 |
| ***E. macrourus* (S)** | **L** | **T0** | 0.683 | 0.134 | 0.987 | **0.000** |
| | | **T1** | - | 0.488 | - | **0.012** |
| | **RC** | **T0** | 0.463 | 0.071 | 1.000 | **0.000** |
| | | **T1** | - | 0.483 | - | **0.020** |
| | **FR** | **T0** | 0.848 | **0.001** | **0.000** | **0.000** |
| | | **T1** | - | **0.037** | - | **0.032** |
| | **Soil** | **T0** | - | - | **0.011** | 0.511 |
| | | **T1** | - | - | - | 0.119 |
| ***B. pumpellianus* (S)** | **L** | **T0** | 0.760 | 0.956 | 0.412 | **0.000** |
| | | **T1** | - | 0.808 | - | **0.000** |
| | **RC** | **T0** | 0.999 | 0.636 | **0.084** | **0.000** |
| | | **T1** | - | 0.606 | - | **0.020** |

**Table 4-5. Cont'd.**

| Plant Species | Tissue | Treatment | $\delta^{13}$C (‰, VPDB) | | $\delta^{15}$N (‰, AIR) | |
|---|---|---|---|---|---|---|
| | | | T1 | T2 | T1 | T2 |
| *B. pumpellianus* (S) | FR | T0 | 1.000 | 0.651 | **0.003** | **0.000** |
| | | T1 | - | 0.641 | - | **0.000** |
| | Soil | T0 | - | - | 0.900 | 0.218 |
| | | T1 | - | - | - | 0.879 |
| *B. pumpellianus* (RC) | L | T0 | NA | 0.993 | NA | 0.889 |
| | | T1 | - | NA | - | NA |
| | RC | T0 | NA | 0.678 | NA | 0.889 |
| | | T1 | - | NA | - | NA |
| | FR | T0 | NA | 0.930 | NA | 0.430 |
| | | T1 | - | NA | - | NA |
| | Soil | T0 | - | - | NA | 0.811 |
| | | T1 | - | - | - | NA |
| *F. altaica* (RC)* | L | T0 | 0.136 | NA | 0.062 | NA |
| | | T1 | - | NA | - | NA |
| | RC | T0 | 0.855 | NA | 0.127 | NA |
| | | T1 | - | NA | - | NA |
| | FR | T0 | 0.098 | NA | **0.014** | NA |
| | | T1 | - | NA | - | NA |
| | Soil | T0 | - | - | 0.129 | NA |
| | | T1 | - | - | - | NA |

**L: Leaf; RC: Root crown; FR: Fine root.**
**T0: ambient $CO_2$; T1: $CO_2$ = 800 ppm; T2: $CO_2$ = 1000 ppm.**
**(S): grown from seed; (RC): grown from root crown.**
**Values in boldface font are statistically significant ($p \leq 0.05$).**
**NA: comparison of means not conducted (lack of sample or replicates < 3).**
***Results were obtained using the Independent-samples t-test, due to the lack of data for T2.**

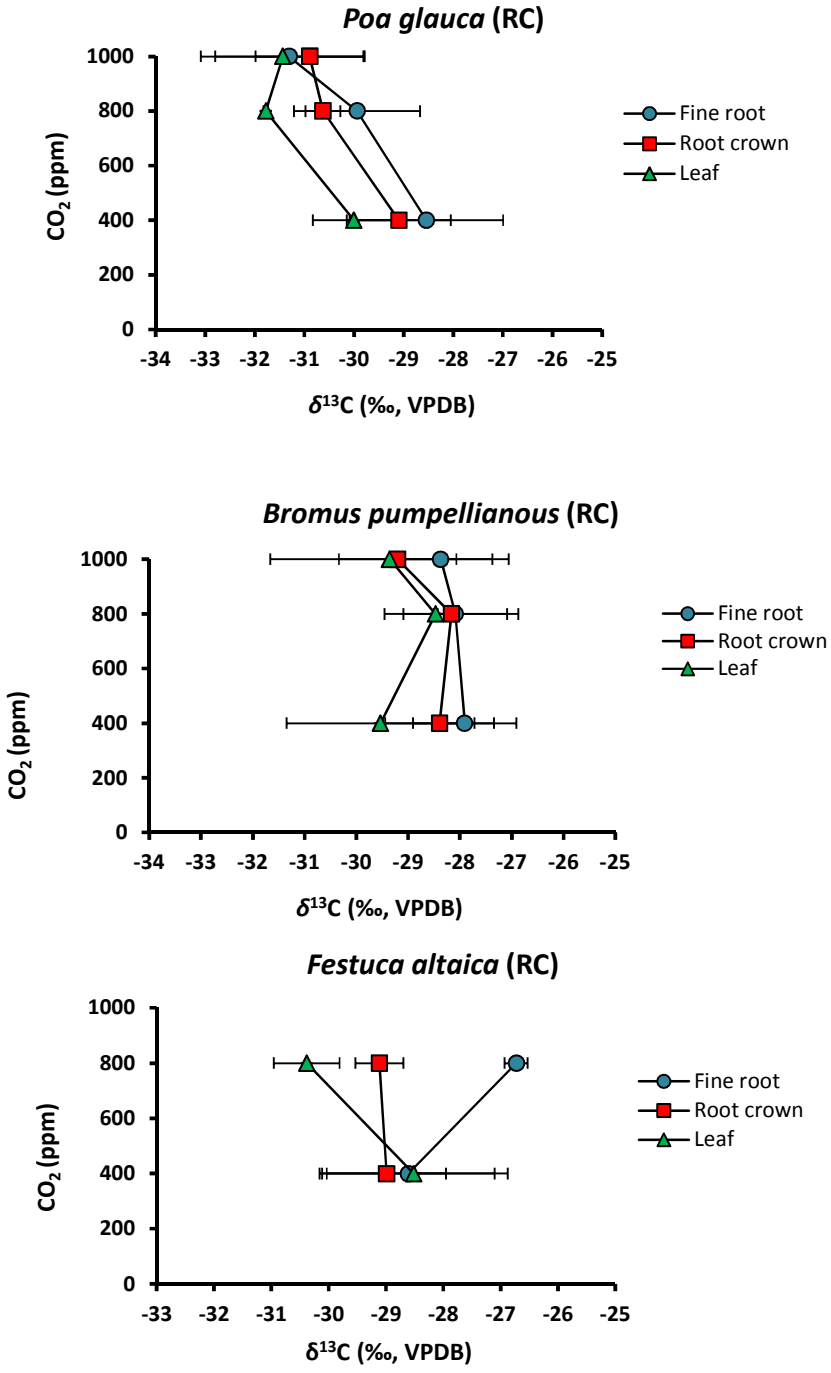


**Figure 4-2: Change in $\delta^{13}C$ of mature plants with $CO_2$ enrichment (ambient, 800 and 1000 ppm). Averages ± SD (n = 6-9) are illustrated.**

### 4.3.3 Nitrogen Isotopic Results

#### 4.3.3.1 Intra-plant Variation in $\delta^{15}N$ within $CO_2$ Treatments

The average $\delta^{15}N$ for the plant parts (fine root, root crown and leaf) of the various species grown from seed and root crown under different $CO_2$ treatments are summarized in Table 4-2. Individual N isotopic results are provided in Appendix K. No root crowns of *F. altaica* were available for experiment T2.

For all three different treatments of $CO_2$ and for all species examined, leaves have higher average $\delta^{15}N$ than fine roots and root crowns, except for *P. glauca* (S) in T0 and *E. macrourus* (S) in T1, which have slightly positive values of $\Delta^{15}N_{RC\text{-}L}$ and/or $\Delta^{15}N_{FR\text{-}L}$ (Table 4-6). Average $\Delta^{15}N_{RC\text{-}L}$ ranges from –4.1 ± 0.8 ‰ to –0.5 ± 0.6 ‰ and average $\Delta^{15}N_{FR\text{-}L}$ ranges from –3.0 ± 2.0 ‰ to –0.1 ± 0.6 ‰ (Table 4-6). These differences are not statistically significant in all cases (Table 4-7), but show a general pattern of higher $\delta^{15}N$ in leaves than fine roots and root crowns.

**Table 4-6: Average $\Delta^{15}N_{RC-L}$ and $\Delta^{15}N_{FR-L}$ for each $CO_2$ treatment.**

| Treatment | Plant Species | $\Delta^{15}N_{RC-L}$ | $\Delta^{15}N_{FR-L}$ |
|---|---|---|---|
| | | (‰, AIR) | |
| T0 | *P. glauca* (S) | +0.8 (± 0.7)* | +0.6 (± 1.1) |
| | *P. glauca* (RC) | −2.4 (± 1.7) | −1.5 (± 0.7) |
| | *E. macrourus* (S) | −0.5 (± 0.6) | −1.6 (± 0.8) |
| | *B. pumpellianus* (S) | −2.0 (± 0.5) | −1.7 (± 0.8) |
| | *B. pumpellianus* (RC) | −2.7 (± 1.0) | −1.5 (± 0.9) |
| | *F. altaica* (RC) | −1.5 (± 1.2) | −0.5 (± 0.7) |
| T1 | *P. glauca* (S) | −1.2 (± 0.9) | 0.0 (± 0.8) |
| | *P. glauca* (RC) | −2.2 (± 2.1) | −1.1 (± 2.0) |
| | *E. macrourus* (S) | −0.8 (± 0.7) | +0.4 (± 2.5) |
| | *B. pumpellianus* (S) | −1.3 (± 1.0) | −0.7 (± 0.7) |
| | *B. pumpellianus* (RC) | −2.9 (± 0.6) | −2.3 (± 1.4) |
| | *F. altaica* (RC) | −2.1 (± 0.3) | −0.1 (± 0.6) |
| T2 | *P. glauca* (S) | −1.3 (± 0.8) | −0.9 (± 0.7) |
| | *P. glauca* (RC) | −4.1 (± 0.8) | −2.7 (± 0.7) |
| | *E. macrourus* (S) | −1.4 (± 1.0) | −2.0 (± 0.5) |
| | *B. pumpellianus* (S) | −3.0 (± 0.4) | −1.2 (± 0.5) |
| | *B. pumpellianus* (RC) | −2.9 (± 3.4) | −3.0 (± 2.0) |
| | *Festuca altaica* (RC) | - | - |

**T0: ambient $CO_2$; T1: $CO_2$ = 800 ppm; T2: $CO_2$ = 1000 ppm.**
**(S): grown from seed; (RC): grown from root crown.**
***Values in parentheses are SD of replicates (n = 9 for seedlings, and n = 3 for plants grown from root crowns).**

**Table 4-7: One-way ANOVA *p*-value results for $\delta^{15}$N differences among plant tissues for each $CO_2$ treatment.**

| Plant Species | | Treatment | | | | | |
|---|---|---|---|---|---|---|---|
| | | T0 | | T1 | | T2 | |
| | Tissue | RC | FR | RC | FR | RC | FR |
| ***P. glauca*** **(S)** | **L** | 0.369 | 0.538 | 0.503 | 0.977 | 0.231 | 0.454 |
| | **RC** | - | 0.946 | - | 0.596 | - | 0.885 |
| ***P. glauca*** **(RC)** | **L** | 0.330 | 0.605 | 0.197 | 0.596 | **0.003** | **0.021** |
| | **RC** | - | 0.843 | - | 0.620 | - | 0.227 |
| ***E. macrourus*** **(S)** | **L** | 0.685 | **0.028** | 0.769 | 0.941 | **0.002** | **0.000** |
| | **RC** | - | 0.152 | - | 0.567 | - | 0.199 |
| ***B. pumpellianus*** **(S)** | **L** | **0.001** | **0.003** | 0.092 | 0.459 | **0.000** | **0.003** |
| | **RC** | - | 0.813 | - | 0.591 | - | **0.000** |
| ***B.s pumpellianus*** **(RC)** | **L** | **0.011** | 0.120 | NA | NA | 0.144 | 0.131 |
| | **RC** | - | 0.200 | - | NA | - | 0.997 |
| ***F. altaica*** **(RC)** | **L** | 0.466 | 0.920 | **0.001** | 0.965 | NA | NA |
| | **RC** | - | 0.679 | - | **0.001** | - | NA |

**L: Leaf; RC: Root crown; FR: Fine root.**
**T0: ambient $CO_2$; T1: $CO_2$ = 800 ppm; T2: $CO_2$ = 1000 ppm.**
**(S): grown from seed; (RC): grown from root crown.**
**Values in boldface font are statistically significant ($p \leq 0.05$).**
**NA: comparison of means not conducted (lack of sample or replicates < 3).**

#### 4.3.3.2 $\delta^{15}$N Variations among $CO_2$ Treatments

The $\delta^{15}$N of the seedlings increases with $CO_2$ enrichment (Fig. 4-3). *P. glauca* (S) shows significant $^{15}$N enrichment from T0 to T1 for leaves and fine roots while *B. pumpellianus* and *E. macrorous* show such enrichment for both T0 to T1 and T0 to T2 for all plant parts (Table 4-5). The rhizosphere soil of the seedlings does not show a significant change in $\delta^{15}$N with $CO_2$ enrichment (Table 4-5). In contrast to seedlings, the $\delta^{15}$N of the mature plants decreases from T0 to T1 (Fig. 4-4) followed by an increase in $\delta^{15}$N from T1 to T2 for *P. glauca* and *B. pumpellianus* (Fig. 4-4). The change in $\delta^{15}$N of rhizosphere soil follows that of plant parts. None of these differences, however, are statistically

significant (Table 4-5). No significant or consistent variation in $\Delta^{15}N_{other\ plant\ part\text{-}leaf}$ is observed with $CO_2$ enrichment for either seedlings or mature plants (Table 4-6).

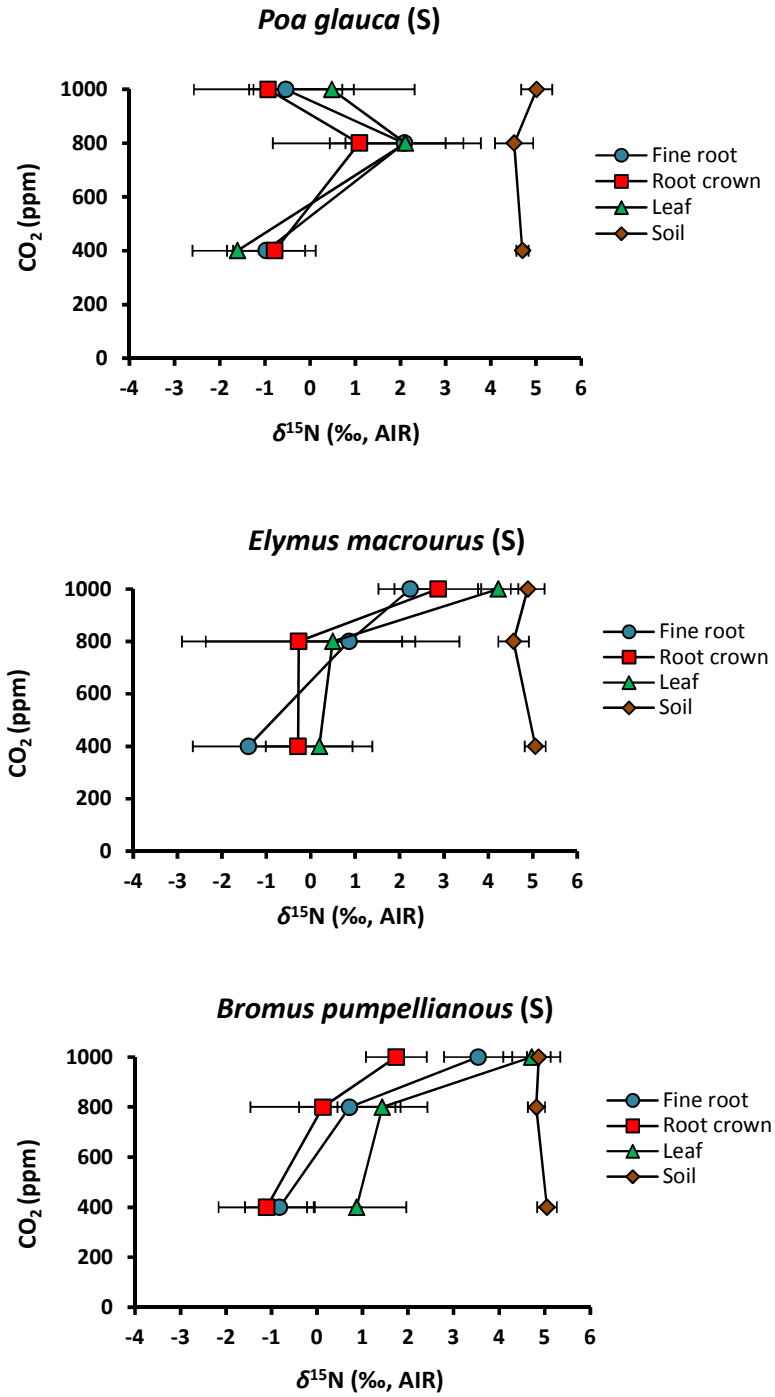


**Figure 4-3: Change in $\delta^{15}N$ of seedlings and rhizosphere soil with $CO_2$ enrichment (ambient, 800 and 1000 ppm). Averages ± SD (n = 6-9) are illustrated.**

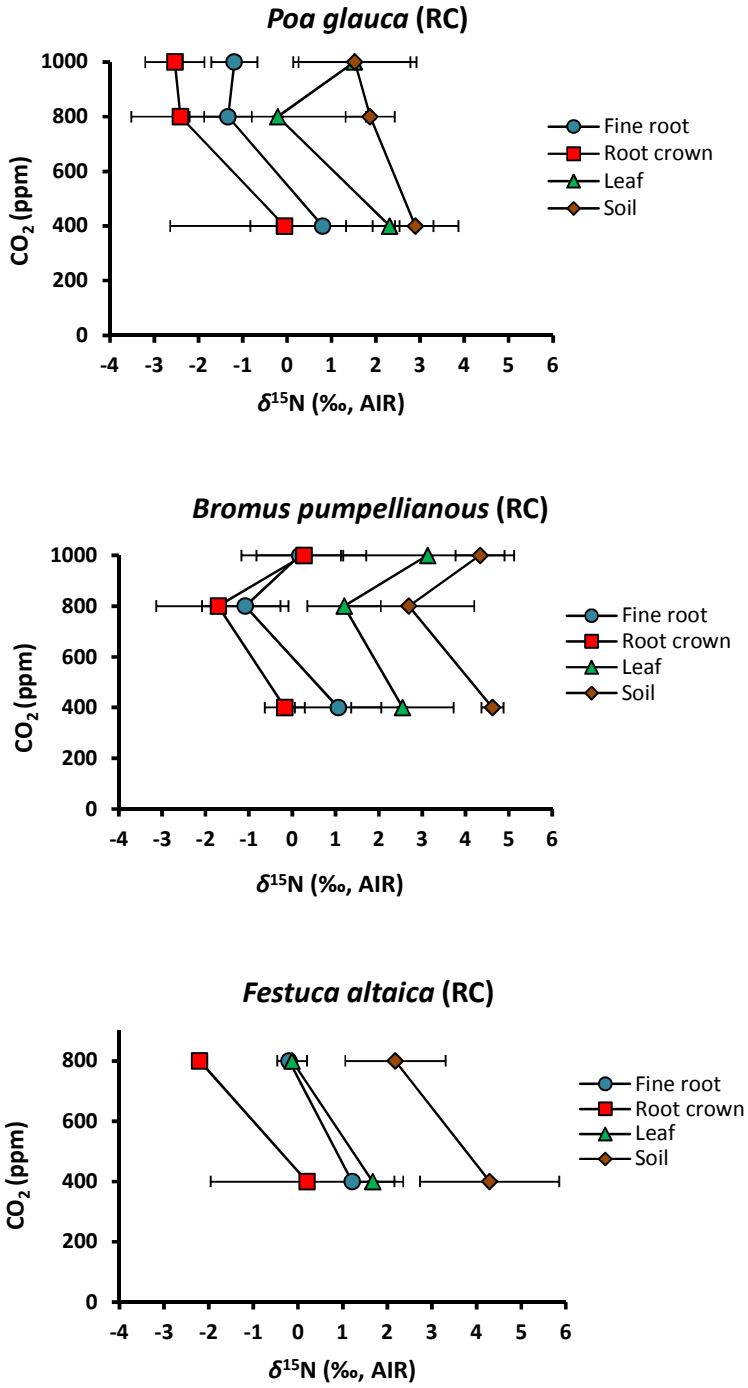


**Figure 4-4: Change in $\delta^{15}N$ of mature plants with $CO_2$ enrichment (ambient, 800 and 1000 ppm). Averages ± SD (n = 6-9) are illustrated.**

### 4.3.4 Carbon and Nitrogen Elemental Results

The average C and N contents for the fine root, root crown and leaf of four different plant species (seedling/mature) examined under three different $CO_2$ treatments are listed in Table 4-8. The C and N contents for individual samples are listed in Appendix L.

#### 4.3.4.1 Intra-plant C and N Variations within Treatments

For all three $CO_2$ concentrations and for most plant species examined, leaves on average have higher C and N contents than fine roots and root crowns (Table 4-9). These differences are statistically significant for almost all seedlings (Tables 4-10, 4-11), but not for all mature plants. *B. pumpellianus* in experiments T1 and T2, and *Elymus macrourus* in experiment T2, have slightly lower nitrogen contents than fine roots and root crowns; these latter differences, however, are not statistically significant (Table 4-11).

**Table 4-8: Average C and N contents of plant parts and rhizosphere soil for different $CO_2$ treatments.**

| Plant Species | Plant part | C (wt. %) | | | N (wt. %) | | |
|---|---|---|---|---|---|---|---|
| | | T0 | T1 | T2 | T0 | T1 | T2 |
| ***E. macrourus* (S)** | **FR** | 41.4 (± 2.1)* | 39.6 (± 1.8) | 42.2 (± 1.4) | 2.0 (± 0.2) | 1.9 (± 0.3) | 1.7 (± 0.3) |
| | **RC** | 37.1 (± 3.1) | 39.0 (± 1.2) | 41.6 (± 0.9) | 2.2 (± 0.2) | 2.0 (± 0.7) | 1.7 (± 0.5) |
| | **L** | 45.7 (± 0.5) | 43.0 (± 0.9) | 45.3 (± 0.4) | 2.7 (± 0.1) | 2.1 (± 0.4) | 1.6 (± 0.3) |
| | **Soil** | - | - | - | 0.1 (± 0.0) | 0.1 (± 0.0) | 0.1 (± 0.0) |
| ***P. glauca* (S)** | **FR** | 40.5 (± 2.6) | 41.0 (± 1.3) | 41.9 (± 1.2) | 2.0 (± 0.1) | 1.8 (± 0.3) | 1.9 (± 0.2) |
| | **RC** | 36.9 (± 3.3) | 35.5 (± 7.8) | 39.7 (± 2.0) | 2.4 (± 0.5) | 1.8 (± 0.2) | 2.4 (± 0.4) |
| | **L** | 45.6 (± 0.9) | 43.2 (± 0.9) | 44.5 (± 2.1) | 3.2 (± 0.3) | 2.5 (± 0.3) | 3.0 (± 0.4) |
| | **Soil** | - | - | - | 0.1 (± 0.0) | 0.2 (± 0.1) | 0.1 (± 0.0) |
| ***B. pumpellianus* (S)** | **FR** | 38.2 (± 5.0) | 38.0 (± 3.6) | 41.6 (± 1.6) | 1.5 (± 0.2) | 1.3 (± 0.1) | 1.1 (± 0.1) |
| | **RC** | 40.1 (± 0.8) | 40.8 (± 1.3) | 43.1 (± 1.1) | 2.5 (± 0.4) | 2.2 (± 0.3) | 1.9 (± 0.2) |
| | **L** | 45.0 (± 0.7) | 43.7 (± 0.3) | 45.7 (± 0.4) | 2.5 (± 0.2) | 2.3 (± 0.2) | 1.8 (± 0.3) |
| | **Soil** | - | - | - | 0.1 (± 0.0) | 0.1 (± 0.0) | 0.1 (± 0.0) |
| ***P. glauca* (RC)** | **FR** | 42.9 (± 3.5) | 39.6 (± 1.5) | 41.1 (± 2.1) | 1.0 (± 0.3) | 1.0 (± 0.2) | 1.4 (± 0.0) |
| | **RC** | 43.2 (± 3.5) | 41.3 (± 3.2) | 41.1 (± 0.5) | 1.1 (± 0.4) | 1.5 (± 0.2) | 1.8 (± 0.2) |
| | **L** | 44.0 (± 1.7) | 41.9 (± 0.1) | 44.6 (± 0.3) | 1.7 (± 0.6) | 2.3 (± 1.0) | 2.8 (± 0.3) |
| | **Soil** | - | - | - | 0.3 (± 0.2) | 0.4 (± 0.1) | 0.4 (± 0.2) |
| ***B. pumpellianus* (RC)** | **FR** | 35.1 (± 3.7) | 33.4 (± 11.4) | 41.6 (± 0.2) | 1.3 (± 0.1) | 1.1 (± 0.3) | 1.3 (± 0.3) |
| | **RC** | 42.2 (± 0.8) | 39.8 (± 2.4) | 42.0 (± 1.4) | 2.6 (± 0.2) | 2.4 (± 0.0) | 2.0 (± 0.3) |

**Table 4-8. Cont'd.**

| Plant Species | Plant | C (wt. %) | | | N (wt. %) | | |
|---|---|---|---|---|---|---|---|
| | part | T0 | T1 | T2 | T0 | T1 | T2 |
| ***B. pumpellianus*** **(RC)** | **L** | 45.4 (± 0.2) | 43.9 (± 1.5) | 46.2 (± 0.6) | 3.0 (± 0.3) | 2.2 (± 0.7) | 2.5 (± 0.5) |
| | **Soil** | - | - | - | 0.1 (± 0.0) | 0.4 (± 0.3) | 0.1 (± 0.0) |
| ***F. altaica*** **(RC)** | **FR** | 34.3 (± 6.8) | 27.5 (± 1.2) | - | 0.9 (± 0.3) | 1.0 (± 0.2) | - |
| | **RC** | 39.1 (± 3.6) | 41.2 (± 1.5) | - | 1.5 (± 0.7) | 1.2 (± 0.0) | - |
| | **L** | 44.1 (± 1.2) | 43.1 (± 0.5) | - | 1.7 (± 0.4) | 1.9 (± 0.2) | - |
| | **Soil** | - | - | - | 0.3 (± 0.0) | 0.3 (± 0.1) | - |

**L: Leaf; RC: Root crown; FR: Fine root.**

**T0: ambient $CO_2$; T1: $CO_2$ = 800 ppm; T2: $CO_2$ = 1000 ppm.**

**(S): grown from seed; (RC): grown from root crown.**

***Values in parentheses are SD of replicates (n = 9 for seedlings, and n = 3 for plants grown from root crowns).**

**Table 4-9: Average difference (*Δ*) in C and N contents between tissues types for each $CO_2$ treatment.**

| Treatment | Plant Species | $\Delta C_{RC-L}$ (wt. %) | $\Delta C_{FR-L}$ (wt. %) | $\Delta N_{RC-L}$ (wt. %) | $\Delta N_{FR-L}$ (wt. %) |
|---|---|---|---|---|---|
| T0 | *P. glauca* (S) | −8.8 (± 3.0) | −5.1 (± 2.4) | −0.8 (± 0.7) | −1.2 ( ± 0.3) |
| | *P. glauca* (RC) | −0.8 (± 1.8) | −1.2 (± 1.8) | −0.6 (± 0.3) | −0.7 (± 0.2) |
| | *E. macrourus* (S) | −8.6 (± 3.2) | −4.3 (± 2.2) | −0.5 (± 0.3) | −0.6 (± 0.2) |
| | *B. pumpellianus* (S) | −4.9 (± 1.1) | −6.8 (± 4.6) | 0.0 (± 0.4) | −1.1 (± 0.3) |
| | *B. pumpellianus* (RC) | −3.2 (± 0.7) | −10.3 (± 3.8) | −0.4 (± 0.2) | −1.7 (± 0.4) |
| | *F. altaica* (RC) | −5.0 (± 4.8) | −9.9 (± 7.8) | −0.2 (± 0.3) | −0.8 (± 0.2) |
| T1 | *P. glauca* (S) | −7.8 (± 7.1) | −2.1 (± 0.9) | −0.7 (± 0.3) | −0.7 (± 0.2) |
| | *P. glauca* (RC) | −0.6 (± 3.1) | −2.3 (± 1.6) | −0.8 (± 1.1) | −1.3 (± 1.1) |
| | *E. macrourus* | −4.0 (± 1.3) | −3.4 (± 1.9) | −0.1 (± 0.4) | −0.1 (± 0.3) |
| | *B. pumpellianus* (S) | −2.9 (± 1.4) | −5.7 (± 3.5) | −0.1 (± 0.2) | −1.0 (± 0.2) |
| | *B. pumpellianus* (RC) | −4.1 (± 0.9) | −10.5 (± 13.0) | +0.2 (± 0.6) | −1.1 (± 0.9) |
| | *F. altaica* (RC) | −1.9 (± 1.2) | −15.6 (± 1.1) | −0.6 (± 0.1) | −0.9 (± 0.1) |
| T2 | *P. glauca* (S) | −4.3 (± 3.5) | −2.3 (± 2.6) | −0.5 (± 0.5) | −1.0 (± 0.5) |
| | *P. glauca* (RC) | −3.5 (± 0.8) | −3.5 (± 2.3) | −1.0 (± 0.1) | −1.5 (± 0.3) |
| | *E. macrourus* (S) | −3.7 (± 1.1) | −3.1 (± 1.7) | +0.2 (± 0.3) | +0.1 (± 0.2) |
| | *B. pumpellianus* (S) | −2.6 (± 1.2) | −4.1 (± 1.7) | +0.1 (± 0.2) | −0.7 (± 0.2) |
| | *B. pumpellianus* (RC) | −4.2 (± 0.9) | −4.6 (± 0.5) | −0.5 (± 0.6) | −1.2 (± 0.3) |
| | *F. altaica* (RC) | - | - | - | - |

**T0: ambient $CO_2$; T1: $CO_2$ = 800 ppm; T2: $CO_2$ = 1000 ppm.**

***Values in parentheses are SD of replicates (n = 9 for seedlings, and n = 3 for plants grown from root crowns).**

**Table 4-10: One-way ANOVA *p*-value results for differences in C contents among plant tissues for each $CO_2$ treatment.**

| Plant Species | | Treatment | | | | | |
|---|---|---|---|---|---|---|---|
| | | T0 | | T1 | | T2 | |
| | Tissue | RC | FR | RC | FR | RC | FR |
| ***P. glauca*** **(S)** | **L** | **0.000** | **0.017** | 0.142 | **0.021** | **0.000** | **0.026** |
| | **RC** | - | 0.090 | - | 0.342 | - | 0.066 |
| ***P. glauca*** **(RC)** | **L** | 0.944 | 0.887 | 0.977 | 0.239 | **0.031** | **0.032** |
| | **RC** | - | 0.988 | - | 0.794 | - | 1.000 |
| ***E. macrourus*** **(S)** | **L** | **0.000** | **0.001** | **0.000** | **0.000** | **0.000** | **0.000** |
| | **RC** | - | **0.011** | - | 0.613 | - | 0.419 |
| ***B. pumpellianus*** **(S)** | **L** | **0.000** | **0.010** | **0.000** | **0.004** | **0.000** | **0.000** |
| | **RC** | | 0.624 | - | 0.137 | - | 0.114 |
| ***B. pumpellianus*** **(RC)** | **L** | **0.031** | 0.083 | NA | NA | **0.003** | **0.002** |
| | **RC** | - | 0.154 | - | NA | - | 0.887 |
| ***F. altaica*** **(RC)** | **L** | 0.411 | 0.081 | 0.181 | **0.000** | NA | NA |
| | **RC** | - | 0.440 | - | **0.000** | - | NA |

**L: Leaf; RC: Root crown; FR: Fine root.**

**T0: ambient $CO_2$; T1: $CO_2$ = 800 ppm; T2: $CO_2$ = 1000 ppm.**

**(S): grown from seed; (RC): grown from root crown.**

**Values in boldface font are statistically significant ($p \leq 0.05$).**

**NA: comparison of means not conducted (lack of sample or replicates < 3).**

**Table 4-11: One-way ANOVA *p*-value results for N contents among plant tissues for each $CO_2$ treatment.**

| Plant Species | | Treatment | | | | | |
|---|---|---|---|---|---|---|---|
| | | T0 | | T1 | | T2 | |
| | Tissue | RC | FR | RC | FR | RC | FR |
| ***P. glauca* (S)** | **L** | **0.006** | **0.000** | **0.000** | **0.000** | **0.010** | **0.000** |
| | **RC** | - | 0.116 | - | 0.997 | - | **0.010** |
| ***P. glauca* (RC)** | **L** | 0.261 | 0.194 | 0.521 | 0.280 | **0.001** | **0.000** |
| | **RC** | - | 0.970 | - | 0.076 | - | **0.045** |
| ***E. macrourus* (S)** | **L** | **0.000** | **0.000** | 0.950 | 0.835 | 0.687 | 0.877 |
| | **RC** | - | 0.380 | - | 0.961 | - | 0.937 |
| ***B. pumpellianus* (S)** | **L** | 0.996 | **0.000** | 0.557 | **0.000** | 0.635 | **0.000** |
| | **RC** | - | **0.000** | - | **0.000** | - | **0.000** |
| ***B. pumpellianus* (RC)** | **L** | 0.103 | **0.000** | NA | NA | 0.342 | **0.019** |
| | **RC** | | **0.001** | - | NA | - | 0.120 |
| ***F. altaica* (RC)** | **L** | 0.892 | 0.211 | **0.009** | **0.002** | NA | NA |
| | **RC** | - | 0.369 | - | 0.280 | - | NA |

**L: Leaf; RC: Root crown; FR: Fine root.**

**T0: ambient $CO_2$; T1: $CO_2$ = 800 ppm; T2: $CO_2$ = 1000 ppm.**

**(S): grown from seed; (RC): grown from root crown.**

**Values in boldface font are statistically significant ($p \leq 0.05$).**

**NA: comparison of means not conducted (lack of sample or replicates < 3).**

#### 4.3.4.2 C and N Variations among $CO_2$ Treatments

There is a consistent lowering of C content of all leaves and fine roots in all species (both seedlings and mature) from experiment T0 to T1, except for the fine roots of *P. glauca* (S) (Figs. 4-5, 4-6), which show a slight but non-significant increase from experiment T0 to T1 (Table 4-12). The carbon content of root crowns shows variable but non-significant changes from T0 to T1 (Table 4-12). From experiment T1 to T2, the C content of almost all seedlings and mature plants increases, but the change is not statistically significant except for leaves of *B. pumpellianus* (S), *E. macrourus* (S) and *P. glauca* (RC) (Table 4-12).

For all plants except *P. glauca* (RC) and *F. altaica* (RC), all plant parts decrease slightly in N content between experiments T0 and T1, but the change is statistically significant only for leaves of seedlings (Table 4-12). N content increases significantly from experiment T1 to T2 in leaves of *P. glauca* seedlings, and decreases significantly in leaves of *B. pumpellianus* and *E. macrourus* seedlings. Rhizosphere soils of all plants show no significant change in N content with $CO_2$ enrichment (Table 4-12).

An increase in atomic C/N with $CO_2$ enrichment is observed for all three different plant parts of seedlings from experiment T1 to T2, except for *P. glauca* (S), which decreases (Table 4-13, Fig. 4-7). This increase is not significant in all cases (Table 4-12). For mature plants, there is no significant or consistent change in atomic C/N with $CO_2$ enrichment (Table 4-12, Fig. 4-7). Almost all leaves and fine roots of *F. altaica* and *P. glauca* show decreasing pattern in atomic C/N with $CO_2$ enrichment. In contrast, leaves and fine roots of *B. pumpellianus* (RC) increase in atomic C/N with $CO_2$ enrichment (Fig. 4-7).

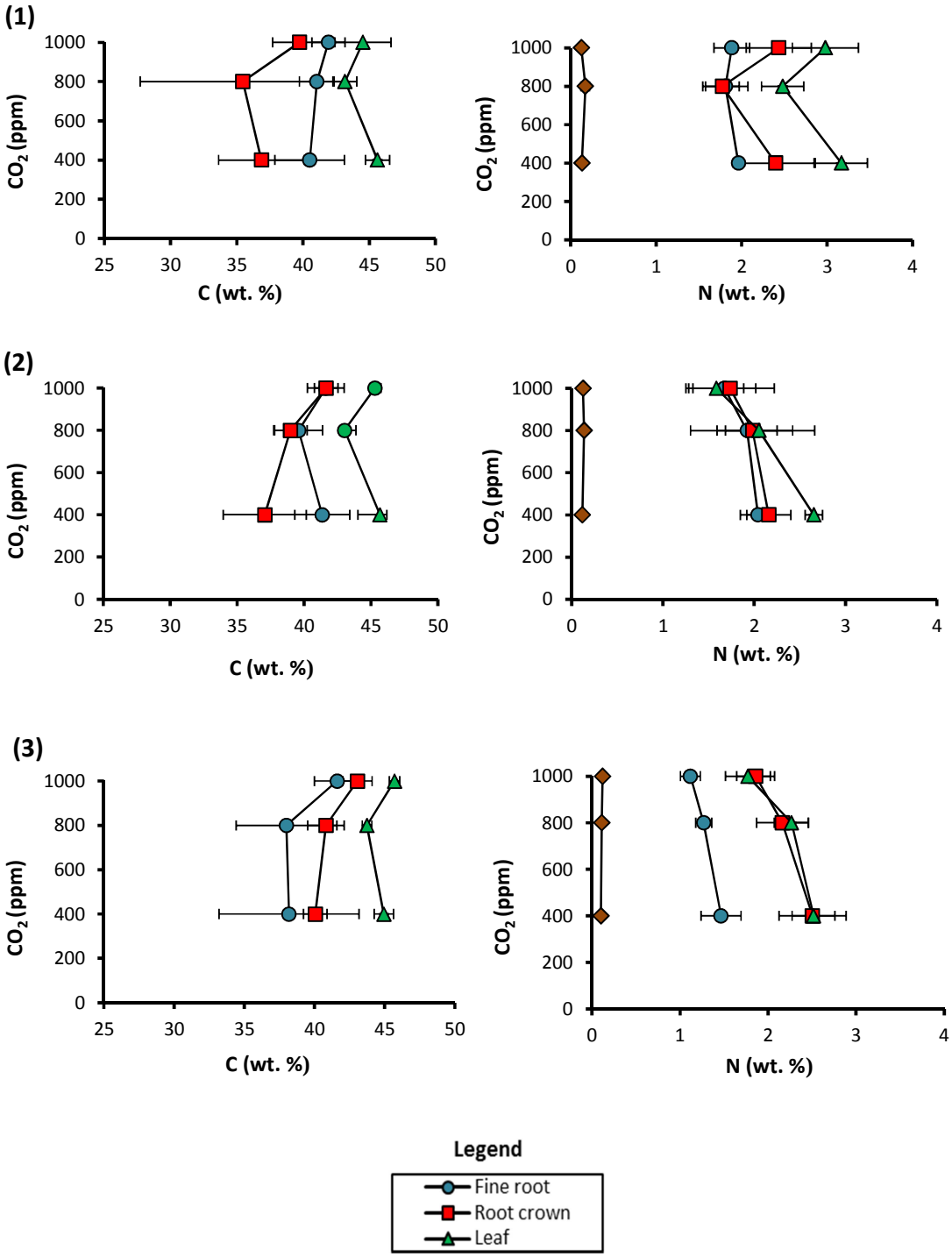


**Figure 4-5: Change in C and N contents of seedling plant parts and rhizosphere soil with $CO_2$ enrichment (ambient, 800 and 1000 ppm): (1) *P. glauca,* (2) *E. macrourus,* and (3) *B. pumpellianus*.**

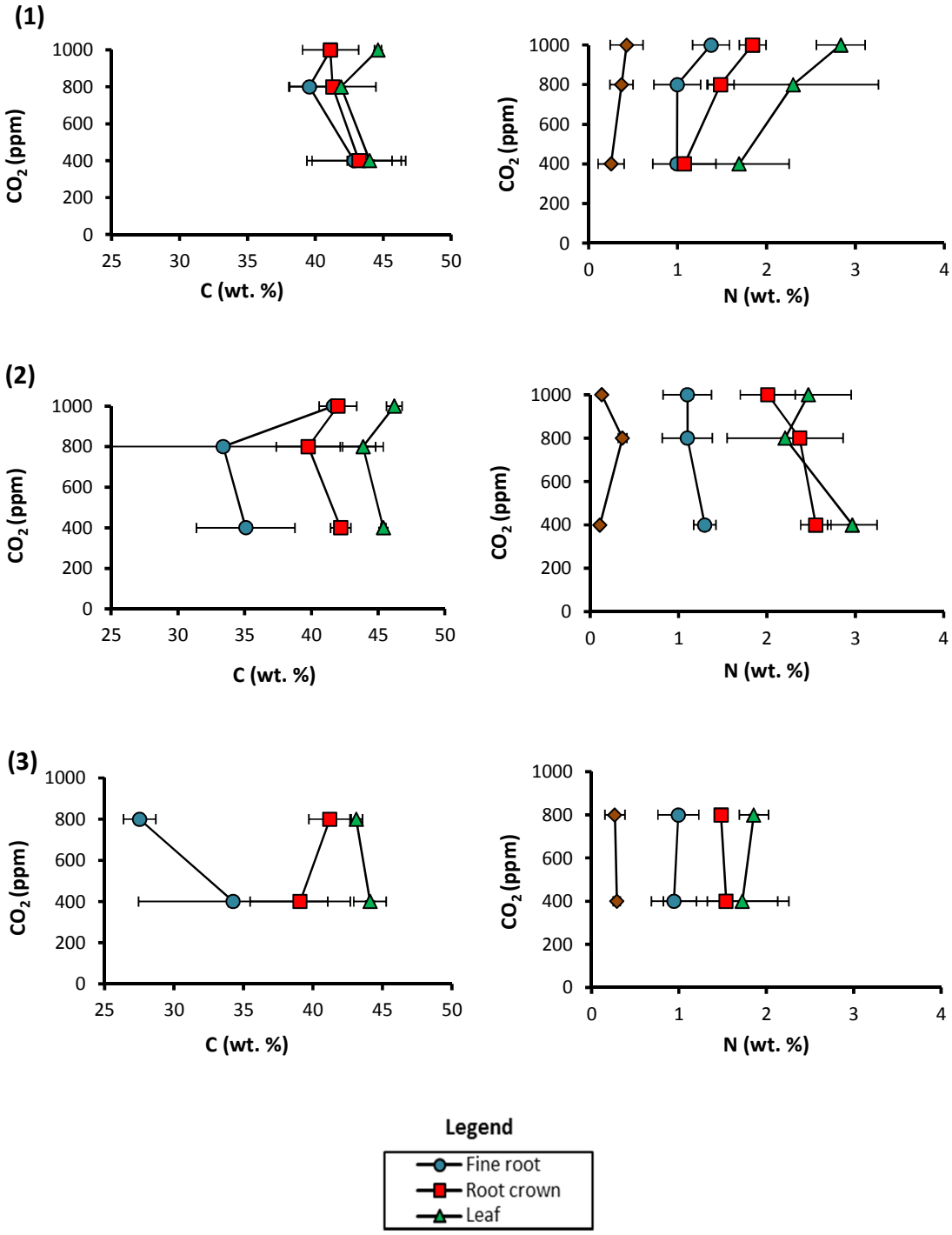


**Figure 4-6: Change in C and N contents of mature plant parts and rhizosphere soil with $CO_2$ enrichment (ambient, 800 and 1000 ppm): (1) *P. glauca*, (2) *B. pumpellianus*, and (3) *F. altaica*.**

**Table 4-12: One-way ANOVA results for differences in C and N contents of different plant parts between $CO_2$ treatments.**

| Plant Species | Tissue | T | C (wt. %) | | N (wt. %) | | Atomic C/N | |
|---|---|---|---|---|---|---|---|---|
| | | | T1 | T2 | T1 | T2 | T1 | T2 |
| *P. glauca* (S) | L | T0 | 0.058 | 0.447 | **0.005** | 0.588 | **0.023** | 0.810 |
| | | T1 | - | 0.318 | - | **0.018** | - | **0.040** |
| | RC | T0 | 0.879 | 0.561 | **0.027** | 0.983 | 0.120 | 0.904 |
| | | T1 | - | 0.256 | - | **0.009** | - | 0.168 |
| | FR | T0 | 0.924 | 0.347 | 0.257 | 0.774 | 0.245 | 0.526 |
| | | T1 | - | 0.533 | - | 0.518 | - | 0.760 |
| | Soil | T0 | - | - | 0.378 | 0.644 | - | - |
| | | T1 | - | - | - | 0.210 | - | - |
| *P. glauca* (RC) | L | T0 | 0.310 | 0.886 | 0.536 | 0.166 | 0.368 | 0.134 |
| | | T1 | - | **0.004** | - | 0.607 | - | 0.707 |
| | RC | T0 | 0.677 | 0.632 | 0.174 | **0.018** | 0.182 | 0.072 |
| | | T1 | - | 0.997 | - | 0.236 | - | |
| | FR | T0 | 0.311 | 0.689 | 1.000 | 0.364 | 0.919 | 0.410 |
| | | T1 | - | 0.736 | - | 0.159 | - | 0.324 |
| | Soil | T0 | - | - | 0.648 | 0.413 | - | - |
| | | T1 | - | - | - | 0.897 | - | - |
| *E. macrourus* | L | T0 | **0.000** | 0.448 | **0.003** | **0.000** | **0.036** | **0.000** |
| (S) | | T1 | - | **0.000** | **-** | **0.028** | - | **0.011** |
| | RC | T0 | 0.288 | **0.006** | 0.738 | 0.192 | 0.321 | **0.035** |
| | | T1 | - | **0.000** | - | 0.554 | - | 0.713 |
| | FR | T0 | 0.107 | 0.549 | 0.730 | **0.045** | 0.942 | **0.028** |
| | | T1 | - | **0.011** | - | 0.344 | - | 0.123 |
| | Soil | T0 | - | - | **0.021** | 0.572 | - | - |
| | | T1 | - | - | - | 0.170 | - | - |
| *B. pumpellianus* | L | T0 | **0.002** | **0.039** | **0.076** | **0.000** | 0.486 | **0.000** |
| (S) | | T1 | - | **0.000** | - | **0.000** | - | **0.000** |
| | RC | T0 | 0.312 | **0.000** | **0.063** | **0.000** | **0.050** | **0.000** |

**Table 4-12. Cont'd.**

| Plant Species | Tissue | T | C (wt. %) | | N (wt. %) | | Atomic C/N | |
|---|---|---|---|---|---|---|---|---|
| | | | T1 | T2 | T1 | T2 | T1 | T2 |
| | | T1 | - | **0.001** | - | 0.108 | - | **0.003** |
| ***B. pumpellianus*** | **FR** | **T0** | 0.994 | 0.137 | **0.034** | **0.000** | **0.003** | **0.000** |
| **(S)** | | **T1** | - | 0.112 | - | 0.120 | - | **0.002** |
| | **Soil** | **T0** | - | - | 0.023 | 0.000 | - | - |
| | | **T1** | - | - | - | 0.023 | - | - |
| ***B. pumpellianus*** | **L** | **T0** | 0.612 | 0.261 | 0.257 | 0.443 | 0.306 | 0.442 |
| **(RC)** | | **T1** | - | 0.426 | - | 0.810 | - | 0.885 |
| | **RC** | **T0** | 0.261 | 0.983 | 0.670 | 0.070 | 0.993 | 0.064 |
| | | **T1** | - | 0.313 | - | 0.278 | - | 0.103 |
| | **FR** | **T0** | 0.994 | 0.185 | 0.640 | 1.000 | 0.738 | 0.285 |
| | | **T1** | - | 0.742 | - | 0.640 | - | 0.733 |
| | **Soil** | **T0** | - | - | 0.685 | 0.486 | - | - |
| | | **T1** | - | - | - | 0.717 | - | - |
| ***F. altaica**** | **L** | **T0** | 0.240 | NA | 0.633 | NA | 0.460 | NA |
| **(RC)** | | **T1** | - | NA | - | NA | - | NA |
| | **RC** | **T0** | 0.396 | NA | 0.528 | NA | 0.351 | NA |
| | | **T1** | - | NA | - | NA | - | NA |
| | **FR** | **T0** | 0.166 | NA | 0.816 | NA | 0.122 | NA |
| | | **T1** | - | NA | - | NA | - | NA |
| | **Soil** | **T0** | - | - | 0.757 | NA | - | - |
| | | **T1** | - | - | - | NA | - | - |

**L: Leaf; RC: Root crown; FR: Fine root.**

**T: Treatment; T0: ambient $CO_2$; T1: $CO_2$ = 800 ppm; T2: $CO_2$ = 1000 ppm.**

**(S): grown from seed; (RC): grown from root crown.**

**Values in boldface font are statistically significant ($p \leq 0.05$).**

**NA: comparison of means not conducted (lack of sample or replicates < 3).**

***Results were obtained using the independent-samples t-test in the absence of data for T2.**

**Table 4-13: Average atomic C/N of different plant parts under different $CO_2$ treatments.**

| Plant Species | Plant part | Atomic C/N | | |
|---|---|---|---|---|
| | | T0 | T1 | T2 |
| *E. macrourus* (S) | FR | 23.8 (± 2.5)* | 24.8 (± 5.2) | 30.5 (± 6.0) |
| | RC | 20.1 (± 1.4) | 25.9 (± 10.2) | 30.3 (± 9.6) |
| | L | 20.1 (± 0.6) | 25.2 (± 4.8) | 34.5 (± 6.5) |
| *P. glauca* (S) | FR | 24.1 (± 1.3) | 27.0 (± 4.5) | 26.3 (± 3.4) |
| | RC | 18.5 (± 3.7) | 23.4 (± 5.2) | 19.4 (± 2.8) |
| | L | 16.9 (± 1.4) | 20.5 (± 2.2) | 17.7 (± 2.6) |
| *B. pumpellianus* (S) | FR | 30.6 (± 2.5) | 34.9 (± 2.2) | 43.9 (± 5.4) |
| | RC | 19.0 (± 2.5) | 22.3 (± 2.8) | 27.3 (± 3.2) |
| | L | 21.0 (± 2.2) | 22.7 (± 2.1) | 30.6 (± 4.3) |
| *P. glauca* (RC) | FR | 54.6 (± 19.0) | 47.6 (± 10.4) | 34.9 (± 2.2) |
| | RC | 50.8 (± 18.4) | 32.6 (± 3.6) | 26.1 (± 2.2) |
| | L | 32.5 (± 10.1) | 23.5 (± 8.1) | 18.5 (± 1.8) |
| *B. pumpellianus (RC)* | FR | 31.6 (± 1.2) | 35.0 (± 3.1) | 38.5 (± 7.4) |
| | RC | 19.3 (± 1.6) | 19.6 (± 0.8) | 24.7 (± 2.9) |
| | L | 18.0 (± 1.7) | 24.2 (± 6.4) | 22.4 (± 4.3) |
| *F. altaica* (RC) | FR | 42.9 (± 3.7) | 33.5 (± 7.5) | - |
| | RC | 32.8 (± 10.3) | 39.3 (± 2.8) | - |
| | L | 30.9 (± 7.3) | 27.2 (± 2.7) | - |

**L: Leaf; RC: Root crown; FR: Fine root.**

**T0: ambient $CO_2$; T1: $CO_2$ = 800 ppm; T2: $CO_2$ = 1000 ppm.**

**(S): grown from seed; (RC): grown from root crown.**

***Values in parentheses are SD of replicates (n = 9 for seedlings, and n = 3 for plants grown from root crowns).**

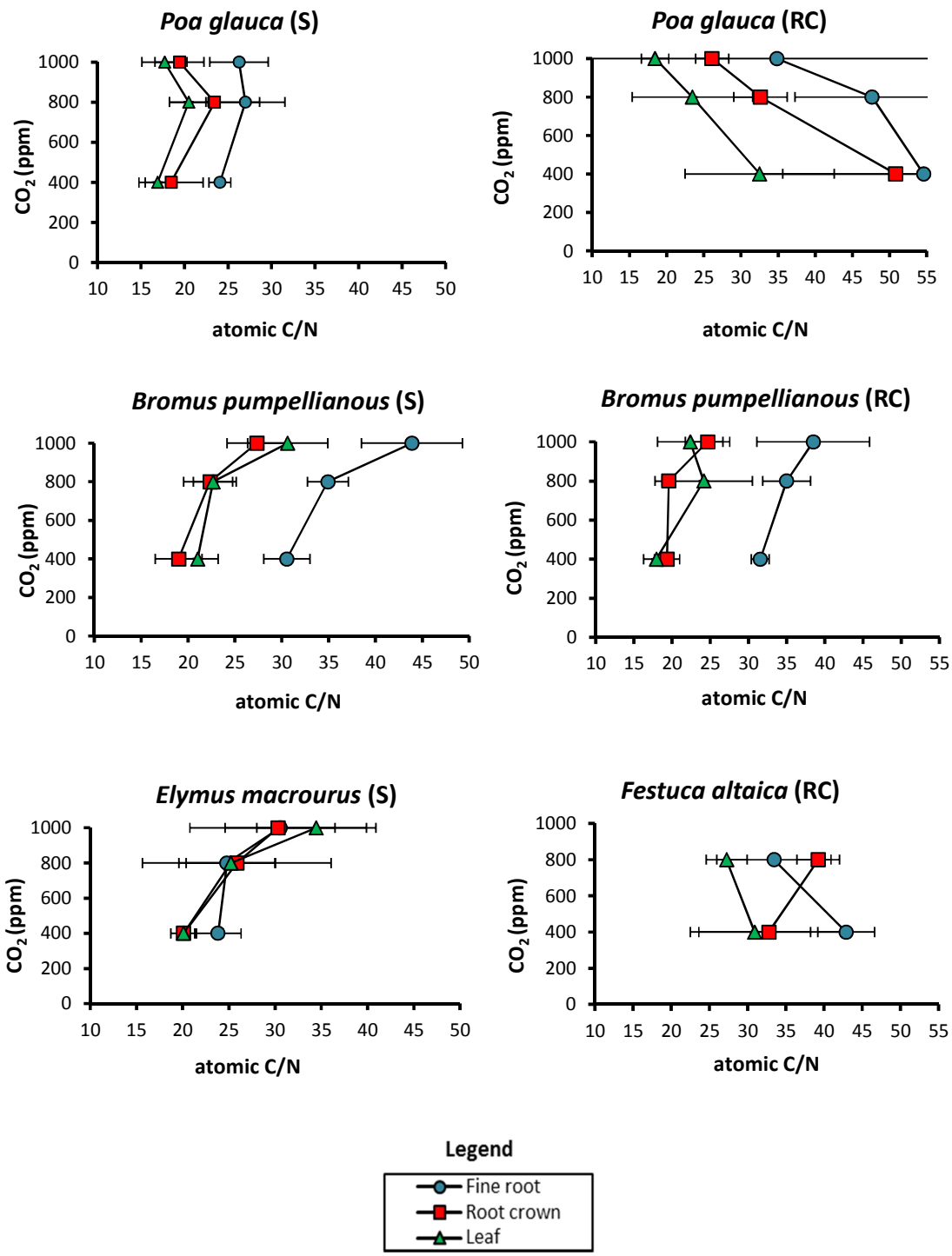


**Figure 4-7: Change in atomic C/N of plant parts with $CO_2$ enrichment (ambient, 800 and 1000 ppm).**

## 4.4 Discussion

### 4.4.1 Intra-plant Variations in $\delta^{13}C$ and $\delta^{15}N$ within $CO_2$ Treatments

Lower $\delta^{13}C$ of leaves than fine roots and root crowns was observed for all species in almost all experiments (T0, T1 and T2) (Table 4-3). The same pattern was also noted for natural plants from Yukon Territory (Chapter 2). Previous studies have also reported more negative $\delta^{13}C$ in photosynthesizing tissues (e.g. leaf) than heterotrophic tissues (e.g. stem, root, and inflorescence) for $C_3$ plants (Brugnoli and Farquhar, 2000; Cernusak et al., 2009; Hobbie and Werner, 2004; Scartazza et al., 1998). The size of the difference recorded in the present study varied among different plant parts, plant species and $CO_2$ treatments (Table 4-3). Different mechanisms have been suggested to be responsible for such intra-plant variability, such as different macromolecular compositions of different tissues, variation in photosynthetic discrimination against $^{13}C$ at the time of tissue development and variation in utilization of day *vs.* night C sources (sucrose) having different C isotopic compositions (Cernusak et al., 2009). Cernusak et al. (2009) suggested that no single factor could explain this pattern, which could be the net result of simultaneous processes.

In contrast to $\delta^{13}C$, higher $\delta^{15}N$ in leaves than fine roots and root crowns was generally observed (Table 4-6). A similar pattern of $^{15}N$-enrichment in leaves than other plant parts (by up to +7 ‰) has been reported for other controlled experiments (Bergersen et al., 1988; Evans et al., 1996; Yoneyama and Kaneko, 1989). This pattern is not universal, however, and can be different, as observed for natural samples in Chapter 2. Contributing factors to such pattern may include variation in plant N sources at different stages of plant development, different patterns of N assimilation of $NO_3^-$ or $NH_4^+$, reallocation of N compounds between organs, and organ-specific efflux of N (Evans, 2001; Szpak et al., 2013).

### 4.4.2 Intra-plant Variations in C and N Contents

The leaves of all seedlings and mature plants in almost all $CO_2$ treatments had higher C and N contents than fine roots and root crowns (Table 4-9). As discussed in Chapter 2, section 2.4.3, temporal variation in N contents of different organs has been reported previously for herbaceous plants (Bausenwein et al., 2001; Choi et al., 2005; Millard, 1988). The present study provided mean June and July (active growing season) conditions for the growth-chamber plants. During the active growing season, plants are characterized by active photosynthesis, leaf expansion and active translocation of N from below-ground parts to leaves and shoots (Choi et al., 2005). At this stage, the developing leaves act as strong sink organs consuming most carbohydrates and nutrients from other organs, and hence show the highest concentration of all nutrients (Hopkins and Hüner, 2009), consistent with our observation of higher C and N contents in leaves than below-ground parts. In Chapter 2, an opposite pattern was observed for natural plant samples collected very late in the growing season, with below-ground parts having higher C and N contents than leaves. Both sets of observations support the idea of temporal translocation of N between different plant organs depending on developmental stage.

### 4.4.3 Carbon Elemental and Isotopic Variations with $CO_2$ Enrichment

The different plant parts of the seedlings (*P. glauca*, *B. pumpellianus* and *E. macrourus*) showed a decrease in $\delta^{13}C$ with increasing $CO_2$ enrichment, but to variable extents (Fig. 4-1). The same pattern has been reported for fossil plants (Van de Water et al., 1994) and modern plants grown under lower gradients of $CO_2$ (LGM to present: 150-350 ppm) (Polley et al., 1993). Schubert and Jahren (2015) reported a global increase of 2.1 ‰ in $\Delta^{13}C$ of $C_3$ plants over the past 30 ka, based on a compilation of previously published $\delta^{13}C$ data for fossil leaves and total organic matter. This response may be related to an increase in plant $\Delta^{13}C$ with $CO_2$ enrichment resulting from an increase in $C_i/C_a$ caused by a change in stomatal conductance and/or more activation of RuBisCO enzyme, as discussed in section 4.1.1. The extent of this response among species may be related to differing abilities to regulate gas exchange (stomatal conductance) in response to

changing $pCO_2$ and to maintain $C_i/C_a$ close to a constant metabolic setpoint (Polley et al., 1993; Wong et al., 1979).

In experiment T1 and coincident with a decrease in $\delta^{13}C$, a small decrease in carbon content was observed for most seedling plant parts (Fig. 4-5), particularly for leaves of *B. pumpellianus* and *E. macrourus* (Table 4-12). In contrast to T1, however, a positive shift in carbon content was observed in experiment T2, even as $\delta^{13}C$ was farther lowered, particularly for *B. pumpellianus* and *E. macrourus* (Table 4-12). Down-regulation of photosynthesis has been suggested as a long-term (acclimation) response of plants to $CO_2$ enrichment (Long et al., 1993; Sims et al., 1998). It is caused by accumulation of non-structural carbohydrate in the cytosol, which then leads to reduced photosynthetic gene expression (Foyer, 1990). It is therefore possible that 60 days was sufficient for the species examined to acclimate to elevated $CO_2$ in T1. The opposite response in T2, however, requires farther discussion.

It has been suggested that the extent of plant acclimation to high $pCO_2$ is much greater in N-limited systems (Ainsworth and Rogers, 2007). If the soil in our experiment could not meet the plant demand for N at elevated $pCO_2$, then N limitation may have stimulated an acclimatory response. Imbalance between plant N demand and supply can cause down-regulation of photosynthesis (Ainsworth and Rogers, 2007; Li et al., 1999). The extent of the plants' response, however, may have been different in T1 and T2 because of a difference in root biomass and thus the ability for N uptake. The root systems developed during T2 were about 2× denser and deeper than observed for T1.

It has been reported that at elevated $pCO_2$, the growth rate of both leaves and roots is increased; however, in N-limited systems leaf growth is less responsive to $CO_2$ enrichment than root growth (Pregitzer et al., 1995; Norby et al., 1992; Ward and Strain, 1999; Zak et al., 1993). Formation of denser and deeper roots at higher $pCO_2$ (T2) would likely increase the plant's ability to search a larger volume of soil and take up more N (Cheng and Johnson, 1998; Rothstein et al., 2000; Zak et al., 2007). This could then reduce the imbalance between N demand and N supply in T2 and attenuate C reduction caused by acclimation. Moreover, an acclimation response of plants to $CO_2$ enrichment

does not always reduce photosynthesis to ambient or lower rates (Ward and Strain, 1999). For example, ponderosa pine trees (*Pinus ponderosa*), which showed strong acclimation-related, down-regulation of photosynthesis in response to high $pCO_2$, still showed higher net photosynthesis (up to 50 %) after six years of treatment than those maintained at ambient $CO_2$ (Tissue et al., 1999). Given this and the small differences in carbon content observed between T0 and T1 *vs.* T0 and T2, we speculate that the seedlings became acclimated to $CO_2$ enrichment after 60 days in both treatments, but the extent of their response differed because of differences in root biomass and the ability to take up N from the system.

In general, none of the mature plants showed a significant change in $\delta^{13}C$ with $CO_2$ enrichment (Table 4-5). *B. pumpellianus* (RC) showed very similar pattern in C content and C isotopic responses to its seedling (Figs. 4-1, -2, -5, -6). *P. glauca* showed a slight difference in the C isotopic response of leaves between seedling and mature plants from T1 to T2, with the mature plants showing a small positive shift relative to seedlings (Fig. 4-1, 4-2). This small difference may indicate a wider variation in the isotopic responses of mature plants that arises from the different ages of root crowns used as replicates (as discussed farther below). The similarity in the pattern of C elemental and isotopic changes with $CO_2$ enrichment between seedlings and mature plants of *P. glauca* and *B. pumpellianus* suggests that for C isotopic studies, results from seedlings can be applied more generally. This idea should be tested for other species.

The non-significant C isotopic response of mature plants to $CO_2$ enrichment, particularly for *P. glauca*, which had significant responses for its seedling, may be related to three factors: (i) stronger acclimation response of mature plants than seedlings to $CO_2$ enrichment because of their different ages and hence different nutrient storage capacities (Turnbull et al., 1998); (ii) lower sensitivity of mature plants than seedlings to higher $pCO_2$ because of the latter's more reliance on new incorporated C for expanding tissues (Dyckmans et al., 1999), and/or (iii) wider variations in isotopic responses of replicates of mature plants within treatments because of their different ages and inherited nutrients. More reliance on C from internal stores by mature plants than seedlings can attenuate C

isotopic responses of mature plants to increased $pCO_2$. Dyckmans et al. (1999), for example, showed that, after six weeks growth under elevated $CO_2$ (700 ppm), a three-year-old beech tree utilized only 56 % of new C in leaves and less than 10 % in roots. Furthermore, root crowns used in the present experiments were collected from different sites along the eastern shoreline of Kluane Lake. Their potentially different ages and hence nutrient storage capacity may have contributed to larger variability in their C isotopic responses to $CO_2$ enrichment (Table 4-2).

Mature plants of *F. altaica* did not show a significant change in $\delta^{13}C$ for different plant parts from T0 to T1. The lack of isotopic data for experiment T2 and comparable seedlings for all treatments, however, limits farther discussion about this species' response to $CO_2$ enrichment.

### 4.4.4 Nitrogen Elemental and Isotopic Variations with $CO_2$ Enrichment

Elevated $CO_2$ had a significant positive effect on $\delta^{15}N$ of all plant parts of *E. macrourus* and *B. pumpellianus* seedlings (Table 4-5). *P. glauca* also showed a significant, positive shift in $\delta^{15}N$ of leaves and fine roots from experiment T0 to T1. This increase points to effects of $CO_2$ enrichment on: (i) soil N cycling and $\delta^{15}N$ of source N (Billings et al., 2004, 2002; Johnson et al., 2000), and/or (ii) the size of plant discrimination against $^{15}N$ at the time of N uptake or N assimilation (Pérez-López et al., 2013).

Elevated $CO_2$ could affect soil N cycling through several mechanisms (see section 4.1.2), of which changes in C allocation from plants to rhizosphere soil have been suggested as the most plausible for mature ecosystems (Zak et al., 2000a). For mature plants, elevated $pCO_2$ could induce an increase in C exudates from fully colonized root systems to soil, which could then overshadow the influence of old soil OM on soil microbial activities (Zak et al., 2000a). Such effects are unlikely in young systems such as short-term controlled growth experiments, however, even if the response of soil microbes to $CO_2$ enrichment over the long term leads to a significant change in N cycling and plant $\delta^{15}N$.

The $\delta^{15}N$ of the bulk rhizosphere soil of all plants did not show significant change with $CO_2$ enrichment (Table 4-5), as also observed by Billings et al. (2002) for bulk soils of plants exposed to $CO_2$ enrichment (550 $\mu$mol C $mol^{-1}$). They noted, however, a significant reduction in soil $NO_3^-$-N and $NH_4^+$-N concentrations and suggested that the observed increase in vegetation $\delta^{15}N$ resulted from a change in soil N cycling and $\delta^{15}N$ of soil N pools. In other words, the absence of a change in bulk soil $\delta^{15}N$ is insufficient evidence for rejecting a change in soil N cycling in response to changing $CO_2$ levels. Bulk soil $\delta^{15}N$ is an integrated measure of all N pools (organic and inorganic), making it difficult to detect isotopic changes in individual N pools (Billings et al., 2002; Hogberg, 1997).

If physiological responses of plants to higher $pCO_2$ are responsible for $^{15}N$ enrichment in seedlings, some change is expected in plant discrimination against $^{15}N$ as $CO_2$ concentrations increase. For barley under elevated $pCO_2$, Pérez-López et al. (2012) observed an increase in nitrate reductase (NR) activity (which is responsible for reduction of $NO_3^-$ to $NO_2^-$ during the first steps of N assimilation), an increase in N uptake, and a reduction in $^{15}N$ discrimination between source-plant N ($\Delta^{15}N$) relative to ambient conditions. They suggested that greater plant growth at elevated $CO_2$ increases plant N demand relative to N supply in soil, thus reducing discrimination against $^{15}N$ during N uptake and enzymatic assimilation (Evans, 2001).

It appears, therefore, that changes in source N isotopic signal and/or plant $^{15}N$ discrimination with $CO_2$ enrichment may explain our observation of higher $\delta^{15}N$ of seedlings in T1 and T2 relative to T0. To discriminate between these possibilities, future studies should include measurement of the abundance and isotopic composition of individual soil N pools and the activity of plant N assimilating enzymes.

Mature plants did not show the change in N isotopic composition as seedlings upon $CO_2$ enrichment. Instead, there was a decrease in $\delta^{15}N$ from T0 to T1 followed by an increase from T1 to T2, but these changes were not statistically significant (Table 4-5). *F. altaica* – with no data for T2 – followed this pattern for T0 to T1. Different physiological and acclimatory responses of plants and soil N cycling to different $pCO_2$ as well as a strong

coupling between these responses, could lead to such nonlinear patterns (Gill et al., 2002). Internal N stores likely have different $\delta^{15}$N than soil; as discussed in section 4.4.3, it is possible that plants during T1 could not acquire sufficient N from the soil to meet their demands, and hence relied more on internal N stores while larger root systems during T2 increased their ability to draw on soil N pools. The denser and more expanded root systems of mature plants could also affect soil microbial processes over a larger volume and hence have stronger influence on soil N cycling (Zak et al., 2000a). Greater reliance of mature plants on N stored in root crowns (Dyckmans et al., 1999) could also lead to different response than that of seedlings. This observation suggests that seedlings of the species examined here may not be a good proxy for mature plants, at least for N isotopic responses to $CO_2$ enrichment.

Seedlings of *E. macrourus* and *B. pumpellianus* experienced a consistent and significant reduction in N (wt. %) with $CO_2$ enrichment, while *P. glauca* showed such a reduction only from T0 to T1 (Fig. 4-5). This observation matches that previously reported for plants exposed to elevated $CO_2$ (Cotrufo et al., 1998; Norby et al., 2001; Pérez-López et al., 2013). The reduction in N content of different plant parts of seedlings, particularly leaves, from T0 to T1 was accompanied by reduction in C content, which was explained by down-regulation of photosynthesis in section 4.4.3. Therefore, accumulation of non-structural carbohydrates alone cannot explain the change in N content. Moreover, loss of Rubisco also cannot explain such a reduction as it was observed in all plant parts, not just leaves (Ainsworth and Long, 2004). As discussed in section 4.4.3, a limited supply of N could cause such a response in seedlings. At 1000 ppm $CO_2$ (T2), N reduction in *E. macrourus* and *B. pumpellianus* seedlings was accompanied by an increase in C content, likely caused by enhanced photosynthesis simulated by $CO_2$ enrichment. This might explain the reduction in N content of these plants. The lack of a concurrent response of C and N uptake could also be another factor causing the N reduction (Dyckmans et al., 1999). Mature plants did not show a statistically significant change in N content with elevated $CO_2$ (Table 4-12). The non-significant increase in N for *P. glauca* (RC) could be related to lower C abundances in T1 and greater N uptake in T2. In summary, the significant increase in atomic C/N with $CO_2$ enrichment shown by all plant parts of

seedlings except for *P. glauca* (S) (Tables 4-12, 4-13; Fig. 4-7) suggests that $CO_2$ enrichment stimulates C fixation and incorporation in plants (e.g. Couture et al., 2012; Lindroth, 2010; Tuchman et al., 2003) at higher rate than N uptake, particularly in N poor systems. Different rates of C and N uptake, which are independently regulated processes, in response to elevated $CO_2$ could explain such observations (Dyckmans et al., 1999).

## 4.5 Conclusion

### 4.5.1 Implication in Paleoecology

Changes in atmospheric $pCO_2$ likely have affected the C and N isotopic compositions of plants during the Quaternary. The need to identify and understand such changes in C and N isotopic baselines is particularly relevant to interpretation of isotopic analyses of animal bone macrofossils, which are increasingly used for paleoecological and paleodietary reconstructions. It has been suggested that plants and ecosystems probably have nonlinear responses to changes in atmospheric $CO_2$ levels (Gill et al., 2002). Therefore, extrapolating from experiments conducted at above-ambient $CO_2$ levels to sub-ambient responses is complicated (Gill et al., 2002; Polley et al., 1993). To the best of our knowledge, no threshold levels of $pCO_2$ needed to engender an isotopic response has been reported for $C_3$ plants under sub-ambient $pCO_2$ gradients. Nonetheless, a strong hyperbolic correlation has been reported between $pCO_2$ levels and $C_3$ plant $\Delta^{13}C$ for different plant tissues (below-ground, above-ground and $nC_{31}$-alkanes) under above-ambient $pCO_2$ (370-4200 ppm), with a linear trend between ambient and 1000 ppm (Schubert and Jahren, 2012).

Based on the response of mature plants, which is likely more representative of natural ecosystems, the present study show a non-significant decrease in plant $\delta^{13}C$ with $CO_2$ enrichment for two very common grasses from the subarctic grassland terrain. If this pattern hold true at lower $pCO_2$, higher $\delta^{13}C$ would be expected for subarctic plants during the late Pleistocene with a shift to more negative values in response to terminal Pleistocene $CO_2$ enrichment. An exact determination of this difference is impossible as the predicted responses could vary depending on plant species, $CO_2$ gradient and the influence and interactions of other environmental factors. Previous studies of other $C_3$

plants at sub-ambient gradient of $pCO_2$ (LGM to present) support our suggestion (Polley et al., 1993; Van de Water et al., 1994).

In contrast, mature plants showed a nonlinear N isotopic response to higher $pCO_2$, with a non-significant decrease in $\delta^{15}N$ from ambient to 800 ppm $CO_2$ to a non-significant positive response from 800 to 1000 ppm $CO_2$. Making predictions for N is more complicated, given such nonlinear response to higher $pCO_2$. Growth-chamber experiments under sub-ambient $pCO_2$ (e.g. 180 ppm) are required to farther interrogate this question.

The results of this study provide an initial understanding of the influence of $pCO_2$ on sub-arctic plant isotopic compositions. Farther investigations employing sub-ambient $pCO_2$ are necessary to test these initial interpretations. A more general caution surrounds application of growth chamber results to real ecosystems. Variations and interactions of other environmental factors across geological timescales could critically co-determine plant responses to changes in $pCO_2$. Hence, the importance of field studies for predicting long-term effects of increasing $pCO_2$ on natural ecosystems should not be underestimated.

### 4.5.2 Implication in Designing Controlled Growth Experiments

This study has demonstrated a similar pattern of change in C isotopic composition with $CO_2$ enrichment for both seedlings and mature plants of two perennial subarctic grass species. Seedlings grown under controlled conditions therefore have potential as a proxy for mature plants in natural ecosystems for the study of C isotopic response of plants to $CO_2$ enrichment. In contrast, the N isotopic response of seedlings grown under controlled conditions at elevated $CO_2$ did not match that of mature plants.

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# Chapter 5

# 5 Conclusions

This chapter is divided into two main parts. First, a summary of main conclusions of this work is provided, along with suggestions for future work. Second, possible causes of the anomalous $^{15}$N-enrichment of woolly mammoth collagen are reviewed using the N isotopic data obtained in this study.

## 5.1 Summary and Future Directions

This thesis investigated the ecology of the late Pleistocene eastern Beringia and its closest possible modern analogues using C and N isotopic analysis of soils, plants and animals. This study has increased our understanding of this region's food web isotopic baselines and factors affecting them using three different approaches: (i) C and N isotopic analysis of modern soils, plants and bones, (ii) C and N isotopic analysis of fossil soils, plants and bones, and (iii) C and N isotopic analysis of soils and plants under controlled-growth experiments. The results have provided C and N isotopic baselines for modern grasslands in the Kluane Lake and Whitehorse areas (Chapter 2) and late Pleistocene eastern Beringia in the same region (Chapter 3). Collectively, the results demonstrate the importance of defining C and N isotopic baselines that account for the effects of climatic and environmental change on nutrient cycling. Many environmental factors contribute to the C and N isotopic signals ultimately acquired and retained by soils and plants at a particular locality (Handley et al., 1994; Schmidt and Stewart, 2003). Distinguishing between past and modern regional isotopic baselines is necessary if we are to correctly interpret the paleoecology and paleodiet of ancient consumers from these regions.

More specifically, the carbon and nitrogen isotopic data presented in Chapter 2 provide a modern baseline for some of the most common grasses also found in late Pleistocene Beringia. All plants analyzed from two main sites in Yukon Territory have an average whole plant $\delta^{13}$C of –27.5 ± 1.2 ‰ and foliar $\delta^{13}$C of –28.0 ± 1.3 ‰, and average whole plant $\delta^{15}$N of –0.3 ± 2.2 ‰ and foliar $\delta^{15}$N of –0.6 ± 2.7 ‰. Next steps should involve

isotopic analysis of a wider variety of plants (including more forbs, shrubs and trees) and investigation of the impact of mycorrhizal fungi associations on plant isotopic compositions in this ecosystem. In addition, C and N isotopic analysis of amino acids from these plants should be conducted to assess isotopic variations at macromolecular levels and their relationships to plant functional group, species and other regional and environmental factors.

The carbon and nitrogen isotopic data presented in Chapter 3 for fossil plants and rodent bones recovered from fossil arctic ground squirrel nests suggest that microbially mediated decay has caused minor changes in the original $\delta^{13}C$ (by ~0-1 ‰) and major changes in the $\delta^{15}N$ of these plant tissues (by ~2-10 ‰). Nonetheless, by (i) coupling the isotopic data for fossil plants and rodent bones, (ii) measuring the modern $\Delta^{15}N$ and $\Delta^{13}C$ spacing between plant and ground squirrels bone collagen, and (iii) accounting for the Suess effect and microbially mediated changes on $\delta^{13}C$ and microbially mediated changes on $\delta^{15}N$, it was possible to estimate the C and N isotopic compositions at the base of the food web in eastern Beringia. A difference ~1.6 ‰ for $\delta^{13}C$ and ~2.5 ‰ for $\delta^{15}N$ was found between modern and late Pleistocene isotopic baselines for this region.

The isotopic results presented in Chapter 3 suggest a possible change in the nature of N cycling in eastern Beringia since the late Pleistocene. Next steps should include additional isotopic analysis of well-dated fossil plants and animal bones from this region along a continuous time series through the terminal Pleistocene to the early and middle Holocene to farther test for the proposed changes in baseline isotopic compositions over time. Such an investigation could provide a more specific view of the changes in C and N isotopic baselines in this evolving ecosystem, and help to identify the most important environmental factors contributing to these shifts. A source of late Pleistocene fossil plant having better preservation would also prove valuable in testing the findings of the present study. Likewise, data from the decomposition experiment showed that plant $\delta^{15}N$ is likely to be much more vulnerable to microbially mediated changes than plant $\delta^{13}C$. The details of these isotopic changes, including the presence and abundance of microbially derived

products in residuals of decomposed plants and their isotopic compositions, should be investigated at the macromolecular level.

The C and N isotopic data presented in Chapter 4 and Appendix I for plants from controlled-growth chamber experiments tested for the effects of $CO_2$ enrichment, dung fertilization and grazing, all of which may have contributed to a change in N cycling in eastern Beringia over time. There is a significant influence of $CO_2$ enrichment on plant seedling $\delta^{13}C$ (decrease) and $\delta^{15}N$ (increase), the extent of which is species-specific. The C isotopic response of mature plants (i.e., grown from root crowns) to $CO_2$ enrichment appeared to be the same as for seedlings, while no consistent isotopic pattern was observed for N, and in both cases the measured differences were not significant. The non-significant changes observed for plants grown from root crowns, which are likely more representatives of natural ecosystems, may somehow be related to different ages for the various root crowns used as replicates in these experiments.

Using these data and assuming the same pattern for plant isotopic response under sub-ambient gradients of $pCO_2$, we predict higher $\delta^{13}C$ (perhaps by a few per mil) for late Pleistocene subarctic plants followed by a shift to lower $\delta^{13}C$ in response to increased $pCO_2$ during the terminal Pleistocene to Holocene transition. The amount of this difference will likely vary depending on plant species, $CO_2$ gradient and the influence of, and interactions with other environmental factors. Prediction of the vegetation N isotopic response to higher $pCO_2$ during the terminal Pleistocene, however, requires new growth chamber studies of using sub-ambient gradients of $pCO_2$. Given the influence of other environmental factors on plant isotopic responses (Gerhart et al., 2012), growth chamber experiments by themselves are also insufficient. Larger-scale investigations (mesocosm to field to landscape) are required in which interactions among multiple variables can be tested for their effects on plant isotopic compositions. In addition, analysis of the N isotopic composition of different N pools in soil and plants at different levels of $pCO_2$ is needed to ascertain whether plant N isotopic responses are related to changes in soil N cycling, plant physiology or both.

The null results obtained for the grazing and fertilization experiments (Appendix I) should be revisited. These experiments should be redesigned using higher levels of organic N fertilization, a longer growth period, and an integrated ecosystem approach, as discussed more fully in Appendix I.

## 5.2 Why did the Woolly Mammoth have higher $\delta^{15}N$ than coeval herbivores?

The woolly mammoth (*Mammuthus primigenius*) was likely a keystone species in sustaining the open pastures and productivity of the Mammoth Steppe Ecosystem (Church, 2013; Owen-Smith, 1987; Putshkov, 2003). During the late Pleistocene, these now extinct megafauna had a wide geographical distribution across the Mammoth Steppe from Western Europe to Siberia and in North America, from Alaska to Yukon (Owen-Smith, 1987; Szpak et al., 2010). The intestinal contents of mummified carcasses of woolly mammoths and isotopic analysis of their tissues suggest a flexible herbaceous diet for these megaherbivores (van Geel et al., 2008), including a wide variety of plants – mainly grasses, herbs and sedges – and to a less degree, mosses and twigs of dwarf shrubs (Kosintsev et al., 2012; van Geel et al., 2008). Moreover, mineral precipitates, clays and sediment (Kosintsev et al., 2010; Mashchenko et al., 2013), and *Coprophilous* fungi indicative of dung consumption (coprophagy) (van Geel et al., 2008, 2011) have been reported in their intestine content. Given a very large body size of woolly mammoths and their short digestive tract (Clauss et al., 2006) with low digestive efficiency, they most probably needed to consume a huge amount of food daily, as has been reported for their modern relatives, the African elephant (up to 150 to 170 kg per d) (Guy, 1975). Such huge demand probably would cause the woolly mammoth to feed on a variety of sources, including those of low quality, particularly during periods of harsh cold and food shortage. Given the key role of these megaherbivores in sustaining Mammoth Steppe Ecosystem (Putshkov, 2003), a good understanding of their foraging ecology and diet is needed to evaluate the potential roles of climatic and environmental change in their extinction.

One of the most interesting puzzles concerning the woolly mammoth is its $^{15}N$-enriched collagen ($\delta^{15}N_{Col} \approx +8$ to $+9$) relative to other herbivores (~+6 to +7 ‰) (e.g. Bocherens, 2003; Fox-Dobbs et al., 2008; Schwartz-Narbonne et al., 2015; see also Fig. 1-3, Chapter 1). Possible explanations for the high $\delta^{15}N_{Col}$ include different dietary choices (Bocherens, 2003; Schwartz-Narbonne et al., 2015), different habitats (Schwartz-Narbonne et al., 2015), physiological differences (Ambrose and DeNiro, 1986; Kuitems et al., 2012), and/or fecal consumption (coprophagy) (van Geel et al., 2008, 2011). We use the N isotopic data obtained in this study for grasses, in addition to previous findings (e.g. Schwartz-Narbonne et al., 2015), to explore the hypothesis that the woolly mammoth diet was enriched in $^{15}N$ relative to the diet of other coeval herbivores.

### 5.2.1 Different Dietary Niches

In natural ecosystems, two or more species cannot inhabit the same ecological niche without exerting strong competition on each other, which can lead to extinction of one of the species (MacArthur and Levins, 1967). It has been hypothesized that separation of food, space or even time are mechanisms that allow coexistence of two species in the same ecological niche (Pianka, 1981; Schoener, 1974). Niche separation or food partitioning has been suggested among extant taxa such as elk, deer and cattle (Chillo et al., 2010; Schoener, 1983; Stewart et al., 2002; Walter, 1991) and extinct late Pleistocene megaherbivores such as proboscideans and ground sloths (Bocherens et al., 2011; Fox-Dobbs et al., 2008; Guthrie, 1982; McDonald and Pelikan, 2006; Rivals et al., 2010; Rivals et al., 2015) that inhabited common habitats. Woolly mammoths living with other herbivores in the same ecosystem may have followed such a strategy through: (i) foraging on specific plant species, plant parts, and/or decomposed or semi-decomposed vegetation, and/or ingesting clay or other mineral matter enriched in $^{15}N$, and/or (ii) occupying specific microhabitats where plants were enriched in $^{15}N$ because of intense dung and urine fertilization.

#### 5.2.1.1 Different Plant Species or Plant Parts

Different $\delta^{15}N$ signals among megaherbivores of Mammoth Steppe Ecosystem may reflect foraging on different plant materials (e.g. different plant functional groups like

grass, lichen and shrub/subshrub; fresh *vs.* dry grass) (Bocherens, 2003). Inter-species and inter-functional group variations in the nitrogen isotopic composition of different life forms (grass, shrub and trees) (Schulze et al., 1994) and species (Michelsen et al., 1996; Nadelhoffer et al., 1996) have been reported for arctic and subarctic ecosystems, but the values of $\delta^{15}$N are generally very low and no plant functional groups or plant species display significant $^{15}$N enrichment. In the present study, the most common modern Yukon grasses (natural conditions, Chapter 2; growth chamber experiments, Chapter 4) also do not show significant $^{15}$N enrichment for specific species, functional groups or plant parts. The $\delta^{15}$N of grasses from two main sites in Yukon Territory ranges from –10 to +7 ‰, with the majority varying from –5 to +5 ‰. Few plants fell in the upper part of this range. Such forage, therefore, would have been insufficient as a source of $^{15}$N-enriched food for the woolly mammoth.

#### 5.2.1.2 Decomposed Plants

The contribution to woolly mammoth diet of decomposed/semi-decomposed plants with higher $\delta^{15}$N but similar $\delta^{13}$C as fresh plants could explain their $^{15}$N-enrichment. The decomposition experiment utilizing common grasses from Beringia (Chapter 3) showed microbially related, significant enrichment of plant tissues in $^{15}$N unaccompanied by a significant change in $\delta^{13}$C. Contribution of decomposed/semi-decomposed plants to mammoths' diet is particularly plausible because it would result in elevated $\delta^{15}$N, without any increase in $\delta^{13}$C, which has been reported to be lower than other herbivores (Szpak et al., 2010).

Both mammoths (Iacumin et al., 2005; Metcalfe et al., 2011; Rountrey et al., 2007) and elephants (Cerling et al., 2004; Codron et al., 2006, 2013; van der Merwe et al., 1988) show seasonal and regional isotopic variations in their diets. Such patterns could reflect local availability of preferred food sources (Codron et al., 2006; Mecalfe et al., 2011). Iacumin et al. (2005) suggested a seasonal pattern in food sources based on $\delta^{15}$N, $\delta^{13}$C and C/N oscillations along woolly mammoth hair samples from one of New Siberian Islands dating to ~42,700 $^{14}$C a BP. They suggested that higher $\delta^{15}$N and lower $\delta^{13}$C and

C/N characterized the mammoth's cold season diet. A similar observation was made from an isotopic study of a tusk from a juvenile woolly mammoth (Rountrey et al., 2007).

Such seasonal isotopic variation in woolly mammoth forage could be explained if decomposed/semi-decomposed plants with high $\delta^{15}$N and low $\delta^{13}$C and C/N were an important food source during the winter season. Senescent above-ground plant parts were probably covered by a thin layer of loess beginning in the fall (Muller, 1967) and then by snow/ice. This covering would have facilitated microbially mediated $^{15}$N-enrichment of the decomposing plant tissue. The tusks and large legs of the woolly mammoth probably gave it a special advantage in gaining access to such plant materials, which would not have been readily available to other herbivores through the snow and soil cover (Putshkov, 2003).

We suggest therefore that woolly mammoths were specialist herbivores, with selective access to food sources such as decomposed plant tissues during the winter. In their study of the intestinal content of a frozen baby woolly mammoth, Kosintsev et al. (2010) noted that the plant detritus was slightly decayed. Whether this occurred before or after ingestion, however, remains an open question.

#### 5.2.1.3 Soil Particles

A few studies have reported the presence of soil and mineral debris in the intestinal tracts of mammoths (Kosintsev et al., 2010; Mashchenko et al., 2013). Large herbivores require mineral matter for proper digestion of plant tissues, as noted in earlier for elephants (Shoshani, 1991) and mammoths (Mashchenko et al., 2013). Leshchinskiy (2001) suggested that Mammoth Steppe vegetation contained insufficient macro- and micro-nutrients (Ca, Mg, Na, Co, Cu, Zn) to meet the needs of large herbivores. The mineral debris content of the large intestine terminal and rectum of a 7-8-month mammoth calf, for example, was estimated at 90 % of its content (Panichev, 1990). Lithophagy (consumption of soil and other mineral matter) was therefore likely an important way of meeting nutritional demands, particularly for mammoths, which had the greatest body mass among all Mammoth Steppe herbivores (Leshchinskiy, 2001). Consideration of this

inorganic debris' contribution to the N-isotopic composition of woolly mammoth tissues is therefore warranted.

Bulk soil analyzed in this study had $\delta^{15}$N of +4 to +5 ‰, which may have contributed to the high $\delta^{15}$N of woolly mammoths. In addition, there are some sites in northern Asia with large accumulations of mammoth remains. These locations are considered as "mineral oases" where large mammals congregated to meet their mineral nutrient needs (Leshchinskiy, 2001). Zeolites, volcanic glass, clay minerals including montmorillonite, and opal are among the most abundant phases at these sites. Clay minerals have a high cation exchange capacity, providing Ca, Mg, Na and even ammonium ($NH_4^+$). Likewise, zeolites are among the most important minerals that can be used as a nutrient and $NH_4^+$ carrier/supplement in fertilizers (Bernardi et al., 2014; Mihajlović et al., 2014). The concentration of large mammals at these mineral 'licks' – and the attendant huge production of urine – could have led to a high rate of ammonia volatilization and thus production of $^{15}$N-enriched $NH_4^+$. The $^{15}$N-enriched $NH_4^+$ could then have been absorbed on clay minerals and/or trapped in zeolites, which were then ingested by herbivores.

### 5.2.2 Different Microhabitats

Use of different microhabitats by species occupying the same ecological niche is another strategy that can reduce strong competition between species and thus enable their long-term coexistence in the same habitat (Pianka, 1974; Schoener, 1974). Elephants, for example, are associated in family groups ranging from 5 to 30 individuals (mature males, females and related off-spring) (Hoppe, 2004). In a study of African elephants in Kenya, it has been observed that elephants have a distinct "home sector" with a "core zone" that they visit more than other areas in the "home sector" (Douglas-Hamilton et al., 2005). Moreover, the elephants' preferred zones in Southern Africa have ample vegetation cover and are located close to water sources (Harris et al., 2008).

If they behaved like their distant relatives, woolly mammoths also probably occupied some specific localities with their family groups. Given the mammoth body mass, the huge quantity of feces likely dropped per day by individuals (up to 100-150 kg of dung

each day by African elephants) (Guy, 1975), and a low digestive efficiency that probably left mammoth feces less depleted of nutrients (Clauss and Hummel, 2005; Clauss et al., 2006), areas occupied by mammoths likely had a higher N content and more open N cycle (see Chapter 2, section 2.4.2.4). Many studies have reported higher plant N contents and N isotopic composition arising from intense herbivory (dung fertilization, grazing) (Augustine and Frank, 2001; Coetsee et al., 2010; Frank et al., 2004; Li et al., 2010). The experiments described in Appendix I, by comparison, showed no significant effect of dung fertilization and grazing on N cycling or plant $\delta^{15}$N. These tests, however, likely were of too short a duration, used insufficiently potent fertilizer (devolatilized, dried cow dung), and/or employed too little fertilizer.

### 5.2.3 A Change in N Isotopic Baseline

Knowing the C and N isotopic baseline of the foodweb is prerequisite to estimating the trophic position of consumers within that ecosystem. These baselines have been established here for both modern (Chapter 2) and Beringian (Chapter 3) grasses from Yukon Territory. These data suggest that late Pleistocene herbivores probably foraged on vegetation with higher $\delta^{15}$N (+2 to +3 ‰) than at the modern time. This higher $\delta^{15}$N baseline likely affected all Beringian herbivores. Nonetheless, it still must be taken into account in any explanation of the $\delta^{15}$N of Beringian woolly mammoths.

### 5.2.4 Synopsis

This study examined different herbivore food sources in eastern Beringia and its closest possible modern remnants to test for variation in $\delta^{15}$N of possible forage. Two groups of materials, decomposed plants and soil/sediment, had the highest $\delta^{15}$N. The elevated $\delta^{15}$N of woolly mammoths relative to other herbivores can be explained by selectively foraging on partially decomposed plants, particularly during harsh winters when fresher plant tissue was not available. N isotopic analysis of amino acids (source amino acids that must come from food *vs.* trophic amino acids, which can be produced by body) in these partially decomposed plant tissues could be used to farther evaluate their contribution to the diet of woolly mammoths (Schwartz-Narbonne et al., 2015). The contribution of soil/sediment to the N-isotopic composition of mammoth tissues may be also important,

but a detailed isotopic investigation of organic and inorganic N in open *vs.* closed Beringian-like soil systems is required before any conclusion can be drawn.

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# Appendices

**Appendix A: Accepted $\delta$ values and elemental concentrations for international and internal standards used in this study.**

| Standard | Material | $\delta^{13}$C (‰, VPDB) | $\delta^{15}$N (‰, AIR) | C (at. %) | N (at. %) |
|---|---|---|---|---|---|
| **IAEA−CH−6**[a] | Sucrose | −10.45 ± 0.03 | – | 42.08 | – |
| **USGS40**[a] | L–glutamic acid | −26.39 ± 0.42 | −4.52 ± 0.1 | 40.78 | 9.51 |
| **USGS41**[a] | L–glutamic acid | +37.63 ± 0.05 | +47.6 ± 0.2 | 40.78 | 9.51 |
| **Keratin** | Keratin | –24.05 ± 0.15[b] | +6.36 ± 0.22[c] | 48.22 ± 1.07[d] | 14.85 ± 0.43[e] |
| **NIST 1547** | Peach leaves | – | +1.98 | – | 2.94[f] |
| **Acetanilide**[g] | N–phenylacetamide | – | – | 71.09 | 10.36 |
| **Low Organic Content Soil** [g] | Soil | – | – | 1.5 ± 0.02 | 0.2 ± 0.005 |
| **High Organic Content Sediment** [g] | Sediment | – | – | 6.5 ± 0.2 | 0.50 ± 0.01 |
| **L-SVEC**[a] | Lithium Carbonate | –46.60 ± 0.2 | | | |

| Standard | Material | $\delta^{13}$C (‰, VPDB) | $\delta^{18}$O | $\delta^{2}$H | |
|---|---|---|---|---|---|
| | | | (‰, VSMOW) | | |
| **NBS-19**[a] | TS-Limestone | +1.95 | +28.60 | - | |
| **NBS-18**[a] | Calcite | –5.0 | +7.20 | - | |
| **WS-1** | Calcite | +0.76 | +26.23 | - | |
| **Suprapur** | Calcite | –35.55 | +13.30 | - | |
| **LSD** | Water | - | –22.57 | –161.8 | |
| **MID** | Water | - | –13.08 | –108.1 | |

**Table. A. Cont'd.**

| Standard | Material | $\delta^{13}C$ (‰, VPDB) | $\delta^{18}O$ | $\delta^{2}H$ |
|---|---|---|---|---|
| | | | (‰, VSMOW) | |
| **EDT** | Water | - | −7.27 | −56.0 |
| **Heaven** | Water | - | −0.27 | +88.7 |

**[a] Accepted $\delta$ values (‰) for international standards are reported as presented on the IAEA website as of March, 2015 (Coplen et al., 2006).**

**[b] C isotopic averaged obtained from 181 keratin analyses by LSIS.**

**[c] N isotopic averaged obtained from 284 keratin analyses by LSIS.**

**[d] Averaged obtained from 28 keratin analyses by the Laboratory for Stable Isotope Science (LSIS).**

[e] **Averaged obtained from 281 keratin analyses by the Laboratory for Stable Isotope Science (LSIS).**

**[f] Certified value (Paul, 2001).**

**[g] Accepted values provided by Elemental Microanalysis Limited.**

## Appendix B: Corrections to elemental composition data.

All plant samples were analyzed using an EA (Costech Analytical Technologies, Valencia, CA, USA) coupled to either a Thermo Finnign Delta$^{PLUS}$ XL or a Thermo Finnign Delta V$^{PLUS}$ IRMS (Thermo Scientific Bremen, Germany) for elemental composition (wt. % C and N). In some of these EA-IRMS analytical sessions, a gradual but systematic shift in peak amplitude for $CO_2$ and $N_2$ gases was observed, which affected the accuracy of C and N concentration data (see section 2.2.2). A correction was therefore applied to these data, calibrated using concentration results obtained using a Fisons 1108 Elemental Analyzer for a representative suite of samples from each EA-IRMS session for which such difficulties were encountered (3-4 samples from the session's start, middle and end).

**Appendix C: Accuracy and precision for standards associated with data presented in Chapter 2, exclusive of those used to generate calibration curves.**

| | | | $\delta^{13}$C (‰, VPDB) | | $\delta^{15}$N (‰, AIR) | | C (wt. %) | | N (wt. %) | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Standard** | **SESSION ID** | **n** | **Mean** | ***SD*** | **Mean** | ***SD*** | **Mean** | ***SD*** | **Mean** | ***SD*** |
| **Keratin** | **CN 12–01**[a] | **9** | –24.07 | 0.11 | | | 48.90 | 0.40 | | |
| | **CN 12–02**[a] | **7** | –24.07 | 0.10 | | | 48.33 | 0.25 | | |
| | **CN 12–03**[a] | **17** | –24.10 | 0.06 | | | 48.61 | 0.89 | | |
| | **CN 14–01**[a] | **9** | –24.03 | 0.08 | | | 47.30 | 0.47 | | |
| | **CN 14–02**[b] | **4** | –24.00 | 0.07 | | | | | | |
| | **CN 14–03**[b] | **10** | –23.99 | 0.10 | | | | | | |
| | **CN 14–04**[a] | **12** | –24.07 | 0.05 | | | 48.00 | 0.21 | | |
| | **CN 14–05**[b] | **10** | –24.09 | 0.04 | | | | | | |
| | **CN 14–06**[b] | **10** | –23.98 | 0.11 | | | | | | |
| | **CO 14–02 (Soil)** | **6** | –24.02 | 0.05 | | | | | | |
| | **CN 14–23 (soil)** | **8** | –24.15 | 0.04 | | | | | | |
| | **NO 12–01** | **9** | | | +6.35 | 0.11 | | | 14.71 | 0.14 |
| | **NO 12–02** | **8** | | | +6.29 | 0.23 | | | 14.62 | 0.57 |
| | **NO 12–03** | **8** | | | +6.35 | 0.34 | | | 14.55 | 1.15 |
| | **NO 13–01** | **6** | | | +6.23 | 0.42 | | | 14.53 | 0.94 |
| | **NO 13–02** | **5** | | | +6.35 | 0.19 | | | 14.36 | 0.54 |
| | **NO 14–01**[b] | **9** | | | +6.34 | 0.10 | | | | |
| | **NO 14–02**[b] | **8** | | | +6.49 | 0.26 | | | | |
| | **NO 14–03** | **6** | | | +6.46 | 0.11 | | | 14.37 | 0.36 |
| | **NO 14–04** | **7** | | | +6.37 | 0.13 | | | 14.41 | 0.39 |
| | **NO 14–05** | **8** | | | +6.38 | 0.20 | | | 14.55 | 0.19 |
| | **NO 14–07** | **7** | | | +6.51 | 0.20 | | | 13.94 | 0.61 |
| | **NO 14–08** | **15** | | | +6.49 | 0.12 | | | 14.31 | 0.38 |
| | **NO 14–19** | **5** | | | +6.37 | 0.12 | | | 13.93 | 0.25 |
| | **NO 14–22 (Soil)** | **7** | | | +6.38 | 0.15 | | | 14.12 | 0.50 |
| | **1108 EA 14–07** | **6** | | | | | 48.64 | 0.53 | 14.86 | 0.12 |
| | **1108 EA 14–08** | **3** | | | | | 46.71 | 1.48 | | |
| | [c]**1108 EA 14–12** | **3** | | | | | 47.36 | 1.05 | 14.57 | 0.32 |
| **IAEA–CH–6** | **CN 12–01** | **3** | –10.37 | 0.01 | | | | | | |

| | | | $\delta^{13}$C (‰, VPDB) | | $\delta^{15}$N (‰, AIR) | | C (wt. %) | | N (wt. %) | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Standard** | **SESSION ID** | **n** | **Mean** | ***SD*** | **Mean** | ***SD*** | **Mean** | ***SD*** | **Mean** | ***SD*** |
| | **CN 12–02** | **3** | –10.41 | 0.02 | | | | | | |
| | **CN 14–01** | **3** | –10.46 | 0.02 | | | | | | |
| | **CN 14–03** | **4** | –10.43 | 0.15 | | | | | | |
| | **CN 14–04** | **4** | –10.47 | 0.04 | | | | | | |
| | **CN 14–05** | **4** | –10.54 | 0.11 | | | | | | |
| | **CN 14–06** | **4** | –10.47 | 0.16 | | | | | | |
| | **CO 14–02 (Soil)** | **3** | –10.43 | 0.09 | | | | | | |
| | **CN 14–23 (Soil)** | **2** | –10.49 | 0.04 | | | | | | |
| **USGS40** | **CN 12–01** | **4** | | 0.05 | | | | | | |
| | **CN 12–02** | **4** | | 0.08 | | | | | | |
| | **CN 12–03** | **6** | | 0.05 | | | | | | |
| | **CN 14–01** | **4** | | 0.03 | | | | | | |
| | **CN 14–03** | **5** | | 0.12 | | | | | | |
| | **CN 14–04** | **5** | | 0.07 | | | | | | |
| | **CN 14–05** | **5** | | 0.02 | | | | | | |
| | **CN 14–06** | **5** | | 0.14 | | | | | | |
| | **CO 14–02 (Soil)** | **3** | | 0.04 | | | | | | |
| | **CN 14–23 (Soil)** | **3** | | 0.06 | | | | | | |
| | **NO 12–01** | **4** | | | | 0.05 | | | | |
| | **NO 12–02** | **4** | | | | 0.07 | | | | |
| | **NO 12–03** | **4** | | | | 0.45 | | | | |
| | **NO 13–01** | **2** | | | | 0.15 | | | | |
| | **NO 13–02** | **3** | | | | 0.08 | | | | |
| | **NO 14–01** | **3** | | | | 0.15 | | | | |
| | **NO 14–02** | **3** | | | | 0.24 | | | | |
| | **NO 14–03** | **2** | | | | 0.30 | | | | |
| | **NO 14–04** | **3** | | | | 0.13 | | | | |
| | **NO 14–05** | **3** | | | | 0.15 | | | | |
| | **NO 14–07** | **3** | | | | 0.07 | | | | |
| | **NO 14–08** | **5** | | | | 0.09 | | | | |
| | **NO 14–19** | **2** | | | | 0.17 | | | | |
| | **NO 14–22 (Soil)** | **3** | | | | 0.24 | | | | |

| | | | $\delta^{13}$C (‰, VPDB) | | $\delta^{15}$N (‰, AIR) | | C (wt. %) | | N (wt. %) | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Standard** | **SESSION ID** | **n** | **Mean** | ***SD*** | **Mean** | ***SD*** | **Mean** | ***SD*** | **Mean** | ***SD*** |
| **USGS41** | **CN 12–01** | **4** | | 0.06 | | | | | | |
| | **CN 12–02** | **4** | | 0.07 | | | | | | |
| | **CN 12–03** | **8** | | 0.09 | | | | | | |
| | **CN 14–01** | **4** | | 0.06 | | | | | | |
| | **CN 14–03** | **5** | | 0.10 | | | | | | |
| | **CN 14–04** | **5** | | 0.09 | | | | | | |
| | **CN 14–05** | **5** | | 0.05 | | | | | | |
| | **CN 14–06** | **5** | | 0.16 | | | | | | |
| | **CO 14–02 (Soil)** | **3** | | 0.15 | | | | | | |
| | **CN 14-23 (Soil)** | **3** | | 0.18 | | | | | | |
| | **NO 12–01** | **4** | | | | 0.64 | | | | |
| | **NO 12–02** | **5** | | | | 0.52 | | | | |
| | **NO 12–03** | **4** | | | | 0.77 | | | | |
| | **NO 13–01** | **2** | | | | 0.25 | | | | |
| | **NO 13–02** | **3** | | | | 0.31 | | | | |
| | **NO 14–01** | **3** | | | | 0.18 | | | | |
| | **NO 14–02** | **3** | | | | 0.13 | | | | |
| | **NO 14–03** | **2** | | | | 0.28 | | | | |
| | **NO 14–04** | **3** | | | | 0.15 | | | | |
| | **NO 14–05** | **3** | | | | 0.28 | | | | |
| | **NO 14–07** | **2** | | | | 0.14 | | | | |
| | **NO 14–08** | **5** | | | | 0.13 | | | | |
| | **NO 14–19** | **2** | | | | 0.03 | | | | |
| | **NO 14–22 (Soil)** | **3** | | | | 0.15 | | | | |
| **NIST 1547** | **EA 14–03** | **4** | | | | | | | 2.79 | 0.01 |
| | **EA 14–04** | **5** | | | | | | | 2.79 | 0.03 |
| | **EA 14–05** | **6** | | | | | | | 2.82 | 0.07 |
| | **EA 14–07** | **4** | | | | | | | 2.80 | 0.01 |
| | **EA 14–08** | **9** | | | | | | | 2.77 | 0.06 |
| | **EA 14–19** | **3** | | | | | | | 2.66 | 0.04 |
| | **1108 EA 14–07** | **4** | | | | | | | 2.78 | 0.02 |

| | | | $\delta^{13}$C (‰, VPDB) | | $\delta^{15}$N (‰, AIR) | | C (wt. %) | | N (wt. %) | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Standard** | **SESSION ID** | **n** | **Mean** | ***SD*** | **Mean** | ***SD*** | **Mean** | ***SD*** | **Mean** | ***SD*** |
| | **1108 EA 14–08** | **3** | | | | | | | 2.80 | 0.01 |
| | **1108 EA 14–10** | **5** | | | | | | | 2.92 | 0.13 |
| | **1108 EA 14–11** | **5** | | | | | | | 2.93 | 0.05 |
| | **1108 EA 14–12** | **2** | | | | | | | 2.79 | 0.01 |
| **Acetanilide** | **1108 EA 14–07** | **12** | | | | | | 1.99 | | 0.32 |
| | **1108 EA 14–08** | **9** | | | | | | 2.01 | | 0.40 |
| | **1108 EA 14–10** | **5** | | | | | | 1.31 | | 0.12 |
| | **1108 EA 14–11** | **5** | | | | | | 0.08 | | 0.07 |
| | **1108 EA 14–12** | **7** | | | | | | 1.05 | | 0.32 |
| **High OC** | **1108 EA 13–05 (Soil)** | **6** | | | | | | 0.06 | | |
| | **1108 EA 14–01 (Soil)** | **6** | | | | | | | | 0.01 |
| **Low OC** | **1108 EA 13–05 (Soil)** | **6** | | | | | | 0.01 | | |
| | **1108 EA 14–01 (Soil)** | **6** | | | | | | | | 0.01 |

**[a] Elemental data corrected, as described in Appendix B.**
**[b] Analytical session repeated using the Fisons 1108 Elemental Analyzer for elemental composition.**
**[c] 1108 EA: Fisons 1108 Elemental Analyzer.**

**Appendix D: $\delta^{13}C$ and $\delta^{15}N$ of all plant parts sampled in 2012 and 2013.**

| Taxonomic | | | $\delta^{13}C$ (‰, VPDB) | | | | | $\delta^{15}N$ (‰, AIR) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Name** | **Site ID** | **Type** | **FR** | **RC** | **S** | **L** | **I** | **FR** | **RC** | **S** | **L** | **I** |
| | | | | | | **2012** | | | | | | |
| *P. glauca* | S12-1 | G | – | −29.3 | −30.0 | −30.0 | −30.7 | – | −0.3 | −2.7 | −1.3 | −1.8 |
| *F. altaica* | S12−1 | G | – | −27.0 | **−26.7** | **−29.4** | −28.8 | – | +0.6 | +0.1 | +1.7 | −1.2 |
| *P. glauca* | S12−2 | G | – | −27.1 | −27.1 | −27.8 | −28.4 | – | −1.0 | +1.2 | **+0.3** | −0.6 |
| *P. glauca* | S12−2 | G | −25.0 | −26.9 | −27.3 | −27.5 | **−29.4** | +2.1 | +2.3 | +1.2 | 1.9 | −1.5 |
| *P. glauca* | S12−2 | G | −26.1 | −26.6 | −27.9 | −27.6 | −28.8 | −0.3 | 1.1 | −0.2 | −1.1 | −1.0 |
| *C. pupurascens* | S12−2 | G | **−26.4** | **−26.3** | −26.0 | −26.1 | −27.1 | −0.3 | −1.0 | −2.1 | −0.9 | −1.9 |
| *P. gormani* | S12−2 | F | – | −26.3 | −27.3 | **−27.6** | −24.2 | – | −1.0 | −0.9 | 2.6 | +1.3 |
| *L. lewisii* | S12−2 | SS | −26.9 | −26.8 | −27.8 | −26.5 | – | +1.0 | **+0.8** | +0.9 | +3.0 | – |
| *L. lewisii* | S12−2 | SS | −24.0 | −25.2 | −25.8 | −25.4 | −25.8 | +1.6 | +1.3 | +0.9 | **+3.0** | +0.2 |
| *E. trachycaulus* | S12−3 | G | −27.9 | −27.3 | −26.7 | −28.6 | −27.9 | +0.4 | +0.7 | **−0.1** | 0.4 | −1.4 |
| *E. trachycaulus* | S12−3 | G | −28.1 | −27.3 | **−26.9** | −27.3 | −29.1 | 0.0 | −1.3 | **+5.9** | −0.3 | 0.5 |
| *F. altaica* | S12−3 | G | −27.5 | −27.8 | −27.8 | −27.3 | −29.2 | +0.3 | −0.5 | −3.4 | −1.5 | −2.1 |
| *P. glauca* | S12−3 | G | −27.7 | −27.3 | −27.1 | −27.1 | −27.9 | 0.0 | −1.2 | −2.3 | −1.7 | +0.2 |
| *P. glauca* | S12−3 | G | **−27.5** | −27.6 | −27.1 | −27.9 | **−28.2** | **−0.3** | −1.7 | −2.2 | −1.2 | −1.4 |
| *F. altaica* | S12−4 | G | −28.8 | −28.9 | −29.3 | −28.6 | −30.0 | +0.4 | +0.9 | −1.9 | −0.6 | **−1.1** |
| *E. trachycaulus* | S12−4 | G | −28.6 | −29.1 | **−29.0** | **−29.4** | −28.5 | +3.3 | +4.9 | +3.3 | +3.3 | +3.2 |
| *E. trachycaulus* | S12−4 | G | −28.74 | −28.95 | **−29.47** | −29.68 | −28.21 | −1.2 | −0.9 | +0.9 | +0.1 | +0.8 |
| *E. trachycaulus* | S12−5 | G | −28.2 | −28.1 | −26.5 | −28.2 | −27.1 | 0.0 | +6.7 | +0.1 | **+0.8** | −1.3 |

**App. D. Cont'd.**

| Taxonomic | | | $\delta^{13}$C (‰, VPDB) | | | | | $\delta^{15}$N (‰, AIR) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Name** | **Site ID** | **Type** | **FR** | **RC** | **S** | **L** | **I** | **FR** | **RC** | **S** | **L** | **I** |
| *E. trachycaulus* | S12–5 | G | −27.6 | −28.4 | −28.8 | −29.3 | −27.5 | +0.5 | +2.7 | +0.6 | +0.9 | +1.2 |
| *E. trachycaulus* | S12–5 | G | −28.1 | **−28.1** | −27.3 | −29.4 | **−27.3** | −11.1 | −3.8 | −7.0 | −4.3 | −0.6 |
| *E. trachycaulus* | S12–5 | G | −28.4 | −28.1 | −27.6 | −28.6 | −25.0 | **−0.3** | +4.1 | −0.6 | +2.2 | +0.9 |
| *C. pupurascens* | S12–5 | G | −26.5 | −26.2 | −25.1 | −27.3 | −26.7 | −1.3 | +2.1 | +0.6 | +1.1 | +2.7 |
| *P. glauca* | S12–5 | G | **−28.3** | −28.7 | −28.6 | −28.9 | −30.0 | **+2.0** | +1.7 | +0.2 | +1.8 | +0.1 |
| *P. glauca* | S12–5 | G | −28.3 | **−28.4** | −28.6 | −29.3 | −30.3 | +0.7 | **+1.2** | +0.2 | +0.9 | +0.8 |
| *E. trachycaulus* | S12–6 | G | −27.1 | −27.2 | −27.4 | −26.0 | **−27.5** | −1.7 | +0.9 | +0.2 | +0.3 | +0.1 |
| *E. trachycaulus* | S12–6 | G | −26.5 | −26.3 | −25.6 | −27.9 | −26.2 | +2.2 | +2.4 | +0.5 | +2.3 | −0.2 |
| *E. trachycaulus* | S12–6 | G | −27.6 | −27.6 | −27.8 | −27.8 | −27.5 | +0.3 | +1.9 | +0.5 | +0.6 | +1.2 |
| *E. trachycaulus* | S12–6 | G | −27.3 | −27.3 | −27.9 | −28.3 | −27.5 | +1.9 | **+2.1** | +0.1 | +2.7 | **+0.5** |
| *C. pupurascens* | S12–6 | G | −25.4 | −25.9 | −25.1 | −25.4 | −26.0 | −1.0 | −1.5 | −1.3 | −1.3 | −3.2 |
| *P. glauca* | S12–6 | G | −26.2 | −26.3 | −27.3 | −27.3 | −27.9 | −1.8 | −1.4 | −2.0 | −1.3 | −1.2 |
| *P. glauca* | S12–6 | G | −25.7 | −26.1 | −25.2 | −26.4 | −25.7 | −1.3 | −2.0 | −2.0 | −1.0 | −0.4 |
| **2013** | | | | | | | | | | | | |
| *E. trachycaulus* | S13–3 | G | −28.3 | −28.6 | −28.7 | −29.4 | −27.0 | −1.4 | −2.4 | −2.0 | −2.3 | −2.1 |
| *E. spicatus* | S13–7 | G | −27.1 | −28.7 | −25.0 | −28.3 | −26.7 | −1.4 | −1.9 | −1.5 | −1.4 | −2.1 |
| *C. filifolia* | S13–8 | SG | −28.5 | −26.9 | −28.5 | −28.2 | −27.7 | −1.2 | −2.0 | −0.7 | 0.0 | −0.9 |
| *E. trachycaulus* | S13–3 | G | −28.3 | −28.7 | −28.2 | −29.1 | −28.4 | −1.9 | −2.5 | −2.8 | −2.1 | −1.9 |
| *C. pupurascens* | S13–2 | G | −27.7 | −28.2 | −26.5 | −27.9 | −27.5 | 0.2 | −0.5 | −1.0 | −0.3 | −0.7 |

**App. D. Cont'd.**

| Taxonomic | | | $\delta^{13}$C (‰, VPDB) | | | | | $\delta^{15}$N (‰, AIR) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Name** | **Site ID** | **Type** | **FR** | **RC** | **S** | **L** | **I** | **FR** | **RC** | **S** | **L** | **I** |
| *B. pumpellianus* | S13–3 | G | –26.6 | –25.7 | **–26.4** | –27.6 | –27.3 | 0.2 | –0.8 | –1.0 | –0.5 | –0.6 |
| *E. spicatus* | S13–7 | G | –27.3 | –28.1 | –25.4 | –27.1 | –25.7 | –1.2 | –2.3 | –3.1 | –1.9 | –1.1 |
| *C. pupurascens* | S13–3 | G | **–26.5** | **–27.0** | –26.2 | –27.4 | **–27.2** | –1.5 | **–1.9** | –3.1 | –2.3 | **–1.6** |
| *A. frigida* | S13–2 | SS | **–28.3** | **–29.3** | **–29.3** | –30.9 | **–27.2** | +5.5 | **+15.3** | +25.4 | +23.4 | +21.2 |
| *A. frigida* | S13–2 | SS | –28.9 | –28.8 | **–29.2** | –30.3 | –28.8 | +12.8 | +6.8 | +22.9 | +18.7 | **+27.4** |
| *E. trachycaulus* | S13–3 | G | –27.8 | –27.9 | –27.2 | –28.9 | –26.5 | –1.3 | –1.9 | –2.4 | **–2.2** | –1.4 |
| *B. pumpellianus* | S13–6 | G | –27.0 | –28.2 | –26.7 | –27.5 | –27.8 | +3.1 | +2.6 | +2.1 | +2.9 | +2.3 |
| *E. rachycaulust* | S13–6 | G | –27.5 | –28.0 | –27.6 | –28.1 | –27.2 | +3.1 | +2.4 | +2.5 | +3.5 | +2.9 |
| *C. pupurascens* | S13–6 | G | –25.6 | –25.9 | –24.7 | –25.5 | –25.6 | +1.4 | +1.0 | –0.8 | +1.4 | +0.9 |
| *P. glauca* | S13–5 | G | –25.8 | –26.5 | –24.4 | –25.8 | –25.4 | +5.4 | +4.1 | 3.9 | +1.8 | +2.3 |
| *P. canescens* | S13–2 | F | –27.7 | –27.6 | –27.0 | –28.7 | –26.5 | –0.8 | –1.0 | **–0.9** | **–0.9** | 0.0 |
| *C. pupurascens* | S13–2 | G | –26.9 | –27.3 | –26.9 | –26.9 | –27.5 | +0.6 | –0.4 | 0.0 | 0.0 | –0.5 |
| *L. ramosissimum* | S13–8 | G | **–26.9** | – | –26.0 | –25.9 | – | +3.7 | – | +4.2 | +7.0 | – |
| *B. glandulosa* | S13–11 | S | – | – | –30.5 | –30.9 | – | – | – | –5.0 | –5.1 | – |
| *P. glauca* | S13–4 | G | –25.8 | –26.4 | –24.4 | –26.6 | **–24.8** | +2.2 | +1.3 | +1.9 | +2.1 | **+1.9** |
| *E. trachycaulus* | S13–3 | G | –27.8 | –29.0 | –29.1 | –29.9 | –28.1 | –1.3 | –1.7 | –0.7 | –1.1 | –0.2 |
| *E. trachycaulus* | S13–4 | G | – | – | –23.5 | –26.5 | –24.3 | – | – | +3.3 | +2.8 | +4.0 |
| *E. trachycaulus* | S13–6 | G | –27.1 | –27.5 | –24.7 | –27.7 | –25.8 | +0.5 | –1.0 | –1.0 | –0.5 | 0.0 |
| *E. spicatus* | S13–7 | G | –27.3 | –26.0 | –24.6 | –28.4 | –25.5 | –0.3 | –2.1 | –3.1 | –2.8 | –1.1 |

**App. D. Cont'd.**

| Taxonomic | | | $\delta^{13}$C (‰, VPDB) | | | | | $\delta^{15}$N (‰, AIR) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Name** | **Site ID** | **Type** | **FR** | **RC** | **S** | **L** | **I** | **FR** | **RC** | **S** | **L** | **I** |
| *F. altaica* | S13–11 | G | –27.7 | –27.5 | –27.5 | –27.6 | –27.8 | –3.9 | –6.3 | –6.2 | –7.2 | –6.2 |
| *A. frigida* | S13–4 | SS | **–27.7** | –27.9 | –26.1 | –29.7 | –25.4 | +1.3 | +3.6 | +1.7 | +4.8 | +3.6 |
| *C. filifolia* | S13–6 | SG | –26.3 | –25.6 | –27.9 | –27.7 | –27.5 | +0.2 | +0.3 | +1.7 | 1.3 | **+3.1** |
| *E. trachycaulus* | S13–3 | G | –27.1 | –27.2 | –26.8 | –28.2 | –24.6 | +3.2 | **+2.6** | +0.6 | +1.1 | +3.8 |
| *B. pumpellianus* | S13–3 | G | –26.8 | –26.2 | –25.5 | –28.4 | –27.1 | –1.2 | –0.8 | –1.6 | –1.5 | –1.8 |
| *P. canescens* | S13–2 | F | –27.2 | –26.2 | –24.7 | –28.6 | –27.4 | **+0.1** | –0.5 | +5.7 | +0.3 | **+0.7** |
| *B. pumpellianus* | S13–3 | G | –26.2 | –26.8 | –25.7 | –27.7 | –27.4 | –0.7 | –1.0 | –1.2 | –0.1 | –0.8 |
| *A. frigida* | S13–6 | SS | –28.3 | –28.2 | –28.2 | **–27.3** | **–26.5** | +1.0 | +1.1 | +1.4 | +2.1 | +3.2 |
| *L. Lewisii* | S13–10 | SS | –26.0 | –27.8 | –27.4 | –27.9 | –27.6 | +2.7 | +2.7 | +0.8 | **+2.9** | +3.0 |
| *C. pupurascens* | S13–2 | G | –26.9 | –27.4 | –26.9 | –27.3 | –27.9 | +1.1 | +0.2 | +0.2 | 0.1 | +1.0 |
| *A. frigida* | S13–7 | SS | –28.8 | –29.8 | –29.2 | –28.2 | **–29.3** | –1.2 | –0.4 | –2.4 | –0.6 | –1.9 |
| *P. glauca* | S13–6 | G | – | – | –27.3 | –28.2 | –27.4 | – | – | –2.5 | –2.6 | –1.7 |
| *C. pupurascens* | S13–3 | G | –26.5 | –24.0 | –24.8 | –25.9 | –26.3 | –1.3 | –1.8 | –3.2 | –2.4 | –3.3 |
| *F. altaica* | S13–11 | G | – | – | **–28.9** | –30.5 | –29.7 | – | – | –5.6 | –4.9 | –4.4 |
| *E. spicatus* | S13–7 | G | –28.5 | –28.9 | –26.9 | –28.4 | –28.4 | –1.1 | –2.5 | –3.3 | –2.2 | 0.0 |
| *P. glauca* | S13–4 | G | –25.9 | **–25.5** | –24.3 | –25.5 | –26.6 | +0.3 | +1.4 | +0.6 | +1.4 | +1.3 |
| *A. frigida* | S13–6 | SS | –28.1 | – | –29.2 | –28.5 | – | 0.0 | – | +1.0 | +2.9 | – |
| *R. idaeus* | S13–13 | S | – | – | –27.4 | **–27.9** | – | – | – | –3.5 | –3.9 | – |
| *B. pumpellianus* | S13–3 | G | –26.6 | –26.4 | –26.9 | –28.1 | –27.5 | –0.2 | –0.9 | –2.2 | –1.3 | –1.7 |

**App. D. Cont'd.**

| Taxonomic | | | $\delta^{13}$C (‰, VPDB) | | | | | $\delta^{15}$N (‰, AIR) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Name** | **Site ID** | **Type** | **FR** | **RC** | **S** | **L** | **I** | **FR** | **RC** | **S** | **L** | **I** |
| *E. spicatus* | S13–7 | G | −27.6 | −28.0 | −26.9 | −28.3 | −26.3 | −1.3 | −2.2 | −4.2 | −2.7 | −2.1 |
| *C. pupurascens* | S13–2 | G | −27.9 | −26.9 | **−26.5** | −27.8 | −26.6 | −2.5 | **−2.5** | −4.7 | −3.6 | −4.4 |
| *R. idaeus* | S13–14 | S | – | – | −32.2 | −30.6 | – | – | – | −1.2 | −0.9 | – |
| *F. altaica* | S13–11 | G | −28.6 | −28.8 | −27.8 | **−27.0** | −28.1 | −3.9 | −4.4 | −9.5 | −8.3 | −8.0 |
| *S. arctica* | S13–15 | S | – | – | **−26.6** | −27.5 | −27.3 | – | – | **−4.0** | −3.5 | −3.2 |

***A. frigidia*: *Artemisia frigida*;**
***B. glandulosa: Betula glandulosa*;**
***B. pumpellianus: Bromus pumpellianus*;**
***C. filifolia*: *Carex filifolia*;**
***C. pupurascens: Calamograstis pupurascens*;**
***E. spicatus*: *Elymus spicatus*;**
***E. trachycaulus: Elymus trachycaulus*;**
***F. altaica*: *Festuca altaica*;**
***L. lewissi*: *Linum Lewisii*;**
***L. ramosissimum: Lepidium ramosissimum*;**
***P. canescens: Plantago canescens*;**
***P. glauca*: *Poa glauca*;**
***P. gormani: Penstemon gormani*;**
***R. idaeus*: *Rubus idaeus*;**
***S. arctica*: *Salix arctica*;**
**FR: Fine root; RC: Root crown; S: Stem; L: Leaf; I: Inflorescence.**
**G: Grass; S: Shrub; SS: Subshrub; SG: Sedge; F: Forb.**
**Boldface denotes average of duplicates.**

**Appendix E: Carbon and Nitrogen contents of all plants according to plant parts and year of sampling.**

| Taxonomic | | | C (wt. %) | | | | | N (wt. %) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Name** | **Site ID** | **Type** | **FR** | **RC** | **S** | **L** | **I** | **FR** | **RC** | **S** | **L** | **I** |
| | | | | | **2012** | | | | | | | |
| *P. glauca* | S12–1 | G | – | 43.3 | 42.9 | 41.4 | 42.1 | – | 1.4 | 0.3 | 0.6 | 1.0 |
| *F. altaica* | S12–1 | G | – | **42.8** | **43.9** | **41.5** | 43.3 | – | 1.5 | 0.1 | 1.1 | 0.4 |
| *P. glauca* | S12–2 | G | – | 41.2 | 41.8 | 41.0 | 38.6 | – | 1.9 | 1.0 | 0.5 | 0.7 |
| *P. glauca* | S12–2 | G | 45.9 | **44.0** | 44.2 | 41.9 | **41.8** | 1.0 | **1.9** | 0.9 | 1.6 | 0.4 |
| *P. glauca* | S12–2 | G | 44.7 | 40.9 | 42.4 | 40.6 | 41.7 | 0.9 | 1.5 | 0.5 | 0.7 | 0.7 |
| *C. pupurascens* | S12–2 | G | 43.6 | **40.5** | 44.1 | 40.3 | 42.3 | 0.5 | 0.9 | 0.3 | 0.3 | 1.3 |
| *P. gormani* | S12–2 | F | – | 43.3 | 45.1 | **45.8** | 45.4 | – | 1.2 | 0.4 | 1.9 | 0.9 |
| *L. lewisii* | S12–2 | SS | 47.2 | 46.8 | 46.0 | 45.5 | – | 1.7 | 2.0 | 1.3 | 4.0 | – |
| *L. lewisii* | S12–2 | SS | 46.4 | 46.0 | 45.2 | 44.4 | 44.4 | 1.5 | 1.7 | 0.9 | **3.5** | 1.0 |
| *E. trachycaulus* | S12–3 | G | 41.7 | 41.3 | 44.2 | 40.0 | 43.4 | 0.8 | 1.2 | 0.2 | 0.8 | 0.5 |
| *E. trachycaulus* | S12–3 | G | **44.9** | 41.8 | **45.2** | 43.5 | 43.4 | **0.7** | 1.1 | **0.2** | 0.5 | 0.7 |
| *F. altaica* | S12–3 | G | 39.9 | 33.0 | 43.4 | 37.5 | 41.4 | 0.5 | 0.9 | 0.1 | 0.5 | 0.4 |
| *P. glauca* | S12–3 | G | 43.7 | 41.8 | 42.4 | 40.3 | **39.7** | 0.6 | 1.4 | 0.4 | 0.5 | 0.7 |
| *P. glauca* | S12–3 | G | 41.2 | 41.0 | 46.1 | 40.8 | 43.6 | **0.8** | 1.1 | 0.2 | 0.9 | 0.3 |
| *F. altaica* | S12–4 | G | 38.4 | 43.0 | 45.3 | 41.0 | 42.4 | 0.9 | 1.3 | 0.2 | 0.5 | 0.5 |
| *E. trachycaulus* | S12–4 | G | **43.9** | 44.6 | **44.7** | **41.6** | 43.7 | **0.8** | 1.2 | 0.2 | 0.5 | 0.9 |
| *E. trachycaulus* | S12–4 | G | 43.5 | 43.1 | **44.6** | 41.6 | 44.2 | 0.9 | 1.2 | 0.2 | 0.4 | 1.4 |

**App. E. Cont'd.**

| Taxonomic | | | C (wt. %) | | | | | N (wt. %) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Name** | **Site ID** | **Type** | **FR** | **RC** | **S** | **L** | **I** | **FR** | **RC** | **S** | **L** | **I** |
| *E. trachycaulus* | S12–5 | G | 42.9 | 43.4 | 45.1 | **41.1** | **43.4** | 1.2 | 1.6 | 0.1 | **0.8** | **0.4** |
| *E. trachycaulus* | S12–5 | G | 43.0 | 42.8 | 45.3 | 38.7 | 44.0 | 1.1 | 1.12 | 0.1 | 0.5 | 0.9 |
| *E. trachycaulus* | S12–5 | G | 41.7 | **43.4** | 44.9 | 40.8 | **44.1** | 1.1 | 1.4 | 0.2 | 1.1 | 0.5 |
| *E. trachycaulus* | S12–5 | G | 42.1 | 40.5 | 43.3 | 41.4 | 44.4 | 1.1 | 1.3 | 0.2 | 0.7 | 0.6 |
| *C. pupurascens* | S12–5 | G | 44.1 | 43.5 | 43.6 | 41.2 | 43.3 | 0.8 | 1.5 | 0.3 | 0.5 | 0.8 |
| *P. glauca* | S12–5 | G | 44.4 | 43.7 | 46.8 | 41.1 | 42.6 | **0.8** | 1.1 | 0.1 | 1.1 | 0.3 |
| *P. glauca* | S12–5 | G | 42.9 | **39.2** | 44.3 | 40.2 | 41.4 | 1.0 | 1.3 | 0.2 | 0.6 | 0.5 |
| *E. trachycaulus* | S12–6 | G | 43.0 | 43.1 | 43.6 | 40.7 | **41.2** | 0.8 | 1.4 | 0.2 | 1.1 | 0.6 |
| *E. trachycaulus* | S12–6 | G | 43.3 | **43.4** | 44.6 | 43.1 | 43.4 | 1.1 | **1.5** | 0.3 | 1.0 | 0.3 |
| *E. trachycaulus* | S12–6 | G | 42.4 | 41.5 | 44.3 | 41.4 | 41.4 | 0.9 | 1.3 | 0.1 | 0.7 | 0.6 |
| *E. trachycaulus* | S12–6 | G | 42.9 | 40.4 | 44.2 | 42.6 | 43.4 | 0.8 | 1.4 | 0.3 | 0.9 | **0.3** |
| *C. pupurascens* | S12–6 | G | 45.5 | 43.2 | 45.1 | 38.6 | 41.4 | 0.6 | 1.1 | 0.3 | 0.4 | 0.4 |
| *P. glauca* | S12–6 | G | 44.4 | 44.1 | 41.5 | 41.7 | 42.9 | 0.8 | 1.4 | 0.6 | 0.8 | 0.9 |
| *P. glauca* | S12–6 | G | 44.3 | 44.2 | 42.2 | 41.4 | 43.2 | 1.09 | **1.34** | 0.54 | 0.79 | 1.07 |
| | | | | | **2013** | | | | | | | |
| *E. trachycaulus* | S13–3 | G | 38.3 | 38.2 | 43.4 | 37.1 | 42.4 | 1.0 | 1.4 | 0.1 | 1.2 | 1.3 |
| *E. spicatus* | S13–7 | G | 35.4 | 40.4 | 43.7 | 41.0 | 42.9 | 0.6 | 1.1 | 0.1 | 1.0 | 0.4 |
| *C. filifolia* | S13–8 | SG | 45.6 | 46.1 | 43.5 | 42.9 | 44.6 | 0.9 | 1.4 | 1.3 | 2.4 | 1.1 |
| *E. trachycaulus* | S13–3 | G | 40.5 | **41.7** | 44.9 | 38.5 | 42.0 | 1.1 | **1.6** | 0.3 | **0.4** | 0.5 |

**App. E. Cont'd.**

| Taxonomic | | | C (wt. %) | | | | | N (wt. %) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Name** | **Site ID** | **Type** | **FR** | **RC** | **S** | **L** | **I** | **FR** | **RC** | **S** | **L** | **I** |
| *C. pupurascens* | S13–2 | G | 40.7 | 41.9 | 44.7 | 39.3 | 43.0 | 0.6 | 0.9 | 0.2 | 1.0 | 0.5 |
| *B. pumpellianus* | S13–3 | G | 41.4 | 42.7 | 43.9 | 41.0 | 39.5 | 0.7 | 1.56 | 0.5 | 1.2 | 0.5 |
| *E. spicatus* | S13–7 | G | 38.7 | 42.4 | 44.4 | 36.6 | 43.3 | 0.7 | 1.3 | 0.2 | 0.5 | 0.4 |
| *C. pupurascens* | S13–3 | G | **42.1** | **43.5** | 45.4 | 38.7 | **39.7** | 0.7 | 1.4 | 0.2 | 0.5 | 0.6 |
| *A. frigida* | S13–2 | SS | 46.4 | 48.5 | 45.3 | 45.6 | 46.7 | 1.0 | 1.8 | **0.7** | 0.6 | 1.3 |
| *A. frigida* | S13–2 | SS | 46.2 | 47.6 | **45.5** | 45.6 | 45.7 | 1.0 | 1.0 | **0.6** | 0.9 | **1.3** |
| *E. trachycaulus* | S13–3 | G | 43.5 | **38.5** | 44.3 | 40.3 | 43.4 | 0.9 | **1.5** | 0.3 | 1.0 | 1.3 |
| *B. pumpellianus* | S13–6 | G | 40.3 | 41.3 | 42.5 | 41.1 | 42.6 | 0.9 | 1.4 | 0.3 | 2.3 | 0.2 |
| *E. rachycaulust* | S13–6 | G | **40.8** | 41.7 | 44.8 | 39.8 | 43.0 | **1.1** | 1.2 | 0.3 | 1.5 | 0.3 |
| *C. pupurascens* | S13–6 | G | 44.5 | **45.8** | 44.1 | 43.2 | 43.0 | 0.8 | **1.5** | 0.3 | 1.6 | 1.0 |
| *P. glauca* | S13–5 | G | 43.2 | 41.5 | 44.5 | 44.5 | 44.2 | 1.4 | 1.4 | 0.8 | 1.1 | 1.0 |
| *P. canescens* | S13–2 | F | 42.4 | 41.6 | 45.1 | 39.7 | 44.2 | 1.2 | 1.0 | 0.2 | 1.5 | 1.2 |
| *C. pupurascens* | S13–2 | G | 41.6 | 39.8 | 43.5 | 40.1 | **42.4** | 0.6 | 1.1 | 0.2 | 1.2 | 0.6 |
| *L. ramosissimum* | S13–8 | G | 43.5 | – | 42.9 | 43.7 | – | 0.9 | – | 2.7 | 3.8 | – |
| *B. glandulosa* | S13–11 | S | – | – | 48.5 | 48.0 | – | – | – | 1.4 | 1.6 | – |
| *P. glauca* | S13–4 | G | 41.8 | 39.5 | 42.6 | 40.5 | 37.7 | 0.8 | 1.1 | 1.4 | 1.0 | 1.1 |
| *E. trachycaulus* | S13–3 | G | **44.4** | **35.5** | 45.3 | 40.2 | 42.6 | **1.0** | **1.5** | 0.2 | 0.7 | 0.3 |
| *E. trachycaulus* | S13–4 | G | – | – | 43.3 | 42.5 | 43.5 | – | – | 0.7 | 0.8 | 1.0 |
| *E. trachycaulus* | S13–6 | G | 39.0 | 44.2 | 45.3 | 42.2 | 43.4 | 0.8 | 1.5 | 0.4 | 0.8 | 0.5 |

**App. E. Cont'd.**

| Taxonomic | | | C (wt. %) | | | | | N (wt. %) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Name** | **Site ID** | **Type** | **FR** | **RC** | **S** | **L** | **I** | **FR** | **RC** | **S** | **L** | **I** |
| *E. spicatus* | S13–7 | G | 36.6 | 39.8 | 45.6 | 38.7 | 44.1 | 0.8 | 1.0 | 0.2 | 0.5 | 0.4 |
| *F. altaica* | S13–11 | G | 42.2 | 40.8 | **45.3** | 41.0 | 39.9 | 0.8 | 1.6 | 0.1 | 1.0 | 0.7 |
| *A. frigida* | S13–4 | SS | **41.2** | 44.1 | 44.9 | 44.0 | 46.1 | **1.6** | 1.6 | 1.4 | 3.0 | 1.8 |
| *C. filifolia* | S13–6 | SG | 44.1 | 47.5 | 42.0 | 41.9 | 43.1 | 0.7 | 0.5 | 0.7 | 0.8 | 1.1 |
| *E. trachycaulus* | S13–3 | G | 39.0 | **40.2** | 44.6 | 42.2 | 40.2 | 0.9 | 1.6 | 0.5 | 0.9 | 1.3 |
| *B. pumpellianus* | S13–3 | G | 40.4 | 39.5 | **43.4** | 39.2 | 41.4 | 1.0 | 2.0 | 0.4 | 0.6 | 0.4 |
| *P. canescens* | S13–2 | F | 42.8 | 41.7 | 43.8 | 37.7 | 43.2 | **0.8** | 1.2 | **0.3** | 1.2 | **0.9** |
| *B. pumpellianus* | S13–3 | G | 30.5 | 41.7 | 43.8 | 40.2 | 41.8 | 0.8 | 1.7 | 0.4 | 1.7 | 0.4 |
| *A. frigida* | S13–6 | SS | 48.4 | 49.9 | 45.0 | **39.0** | 44.5 | 1.5 | 1.1 | 0.7 | **2.1** | 1.4 |
| *L. Lewisii* | S13–10 | SS | 46.2 | 47.1 | 46.6 | 43.0 | 47.4 | 1.2 | 1.5 | 0.5 | **3.1** | 1.4 |
| *C. pupurascens* | S13–2 | G | 43.0 | 38.7 | 43.4 | 34.7 | 42.7 | 0.4 | **0.9** | 0.3 | 0.4 | **0.8** |
| *A. frigida* | S13–7 | SS | 41.9 | 50.4 | 45.4 | 45.3 | 45.7 | 1.1 | 1.2 | 0.8 | 2.3 | 1.4 |
| *P. glauca* | S13–6 | G | – | – | 42.7 | 41.1 | 41.1 | – | – | 1.1 | 0.5 | 1.6 |
| *C. pupurascens* | S13–3 | G | 42.2 | 41.3 | 43.7 | 39.0 | 40.9 | 0.7 | 1.4 | 0.3 | 0.6 | 0.6 |
| *F. altaica* | S13–11 | G | – | – | **44.4** | 43.3 | 41.7 | – | – | **0.2** | 0.4 | 0.5 |
| *E. spicatus* | S13–7 | G | 41.8 | 39.9 | 45.3 | 40.6 | 44.1 | 0.9 | 1.2 | 0.9 | 0.4 | 0.6 |
| *P. glauca* | S13–4 | G | 42.6 | 41.4 | 43.0 | 40.1 | 36.4 | 1.1 | 2.0 | 1.2 | 1.3 | **1.1** |
| *A. frigida* | S13–6 | SS | 49.5 | – | 46.8 | **42.7** | – | 1.0 | – | 1.0 | 2.0 | – |
| *R. idaeus* | S13–13 | S | – | – | 45.4 | **45.6** | – | – | – | **0.8** | 1.8 | – |

**App. E. Cont'd.**

| Taxonomic | | | C (wt. %) | | | | | N (wt. %) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Name** | **Site ID** | **Type** | **FR** | **RC** | **S** | **L** | **I** | **FR** | **RC** | **S** | **L** | **I** |
| *B. pumpellianus* | S13–3 | G | 37.5 | 39.9 | 43.0 | 38.4 | 41.0 | 0.8 | 1.8 | 0.3 | 0.8 | 0.5 |
| *E. spicatus* | S13–7 | G | 39.7 | 40.8 | 45.1 | 41.8 | 43.7 | 1.3 | 1.2 | 0.2 | 0.8 | 0.3 |
| *C. pupurascens* | S13–2 | G | 43.6 | 29.3 | 44.0 | 41.0 | 40.6 | 0.8 | 0.8 | 0.2 | 1.0 | 0.5 |
| *R. idaeus* | S13–14 | S | – | – | **46.3** | 44.2 | – | – | – | **0.6** | 1.9 | – |
| *F. altaica* | S13–11 | G | 43.9 | 41.6 | 45.3 | 41.1 | 41.4 | 0.7 | 1.0 | 0.2 | 0.8 | 0.6 |
| *S. arctica* | S13–15 | S | – | – | 47.7 | **45.3** | **45.8** | – | – | 1.4 | **0.9** | **0.9** |

***A. frigidia*: *Artemisia frigida*;**
***B. glandulosa: Betula glandulosa*;**
***B. pumpellianus: Bromus pumpellianus*;**
***C. filifolia*: *Carex filifolia*;**
***C. pupurascens: Calamograstis pupurascens*;**
***E. spicatus*: *Elymus spicatus*;**
***E. trachycaulus: Elymus trachycaulus*;**
***F. altaica*: *Festuca altaica*;**
***L. lewissi*: *Linum Lewisii*;**
***L. ramosissimum: Lepidium ramosissimum*;**
***P. canescens: Plantago canescens*;**
***P. glauca*: *Poa glauca*;**
***P. gormani: Penstemon gormani*;**
***R. idaeus*: *Rubus idaeus*;**
***S. arctica*: *Salix arctica*;**
**FR: Fine root; RC: Root crown; S: Stem; L: Leaf; I: Inflorescence.**
**G: Grass; S: Shrub; SS: Subshrub; SG: Sedge; F: Forb.**
**Boldface denotes average of duplicates.**

**Appendix F: Foliar atomic C/N of modern plants according to year of sampling.**

| Taxonomic Name | Site ID | Type | Atomic C/N |
|---|---|---|---|
| **2012** | | | |
| *P. glauca* | S12–1 | Grass | 80.5 |
| *F. altaica* | S12–1 | Grass | 43.2 |
| *P. glauca* | S12–2 | Grass | 191.3 |
| *P. glauca* | S12–2 | Grass | 30.5 |
| *P. glauca* | S12–2 | Grass | 71.7 |
| *C. pupurascens* | S12–2 | Grass | 187.8 |
| *P. gormani* | S12–2 | Forb | 28.7 |
| *L. lewisii* | S12–2 | Subshrub | 13.2 |
| *L. lewisii* | S12–2 | Subshrub | 14.8 |
| *E. trachycaulus* | S12–3 | Grass | 60.5 |
| *E. trachycaulus* | S12–3 | Grass | 99.5 |
| *F. altaica* | S12–3 | Grass | 81.0 |
| *P. glauca* | S12–3 | Grass | 95.8 |
| *P. glauca* | S12–3 | Grass | 52.3 |
| *F. altaica* | S12–4 | Grass | 103.8 |
| *E. trachycaulus* | S12–4 | Grass | 99.1 |
| *E. trachycaulus* | S12–4 | Grass | 124.4 |
| *E. trachycaulus* | S12–5 | Grass | 64.0 |
| *E. trachycaulus* | S12–6 | Grass | 88.5 |
| *E. trachycaulus* | S12–6 | Grass | 42.5 |
| *E. trachycaulus* | S12–6 | Grass | 69.9 |
| *C. purpurascens* | S12–6 | Grass | 98.0 |
| *P. glauca* | S12–6 | Grass | 42.4 |
| *P. glauca* | S12–6 | Grass | 74.5 |
| *E. trachycaulus* | S12–7 | Grass | 43.5 |
| *E. trachycaulus* | S12–7 | Grass | 52.9 |
| *E. trachycaulus* | S12–7 | Grass | 71.0 |
| *E. trachycaulus* | S12–7 | Grass | 53.4 |

**App. F. Cont'd.**

| Taxonomic Name | Site ID | Type | Atomic C/N |
|---|---|---|---|
| *C. purpurascens* | S12–7 | Grass | 104.6 |
| *P. glauca* | S12–7 | Grass | 57.8 |
| *P. glauca* | S12–7 | Grass | 61.1 |
| **2013** | | | |
| *E. trachycaulus* | S13–3 | Grass | 36.0 |
| *E. spicatus* | S13–7 | Grass | 48.2 |
| *C. filifolia* | S13–8 | Sedge | 21.2 |
| *E. trachycaulus* | S13–3 | Grass | 102.0 |
| *C. pupurascens* | S13–2 | Grass | 48.2 |
| *B. pumpellianus* | S13–3 | Grass | 41.3 |
| *E. spicatus* | S13–7 | Grass | 92.9 |
| *C. pupurascens* | S13–3 | Grass | 93.9 |
| *A. frigida* | S13–2 | Subshrub | 87.2 |
| *A. frigida* | S13–2 | Subshrub | 58.5 |
| *E. trachycaulus* | S13–3 | Grass | 49.0 |
| *B. pumpellianus* | S13–6 | Grass | 21.3 |
| *E. trachycaulus* | S13–6 | Grass | 31.8 |
| *C. pupurascens* | S13–6 | Grass | 32.5 |
| *P. glauca* | S13–5 | Grass | 47.6 |
| *P. canescens* | S13–2 | Forb | 30.0 |
| *C. pupurascens* | S13–2 | Grass | 39.0 |
| *L. ramosissimum* | S13–8 | Grass | 13.4 |
| *B. glandulosa* | S13–11 | Shrub | 35.4 |
| *P. glauca* | S13–4 | Grass | 46.8 |
| *E. trachycaulus* | S13–3 | Grass | 72.1 |
| *E. trachycaulus* | S13–4 | Grass | 64.4 |
| *E. trachycaulus* | S13–6 | Grass | 62.3 |
| *E. spicatus* | S13–7 | Grass | 90.3 |
| *F. altaica* | S13–11 | Grass | 49.9 |

**App. F. Cont'd.**

| Taxonomic Name | Site ID | Type | Atomic C/N |
|---|---|---|---|
| *E. trachycaulus* | S13–6 | Grass | 62.3 |
| *E. spicatus* | S13–7 | Grass | 90.3 |
| *F. altaica* | S13–11 | Grass | 49.9 |
| *A. frigida* | S13–4 | Subshrub | 17.0 |
| *C. filifolia* | S13–6 | Sedge | 58.9 |
| *E. trachycaulus* | S13–3 | Grass | 57.8 |
| *B. pumpellianus* | S13–3 | Grass | 80.1 |
| *P. canescens* | S13–2 | Forb | 35.5 |
| *B. pumpellianus* | S13–3 | Grass | 28.4 |
| *A. frigida* | S13–6 | Subshrub | 21.5 |
| *L. Lewisii* | S13–10 | Subshrub | 16.4 |
| *C. pupurascens* | S13–2 | Grass | 115.5 |
| *A. frigida* | S13–7 | Subshrub | 23.1 |
| *P. glauca* | S13–6 | Grass | 95.9 |
| *C. pupurascens* | S13–3 | Grass | 77.1 |
| *F. altaica* | S13–11 | Grass | 129.5 |
| *E. spicatus* | S13–7 | Grass | 115.6 |
| *P. glauca* | S13–4 | Grass | 34.9 |
| *A. frigida* | S13–6 | Subshrub | 24.9 |
| *R. idaeus* | S13–13 | Shrub | 29.1 |
| *B. pumpellianus* | S13–3 | Grass | 55.2 |
| *E. spicatus* | S13–7 | Grass | 62.5 |
| *C. pupurascens* | S13–2 | Grass | 47.4 |
| *R. idaeus* | S13–14 | Shrub | 26.7 |
| *F. altaica* | S13–11 | Grass | 57.0 |
| *S. arctica* | S13–15 | Shrub | 56.8 |

**Appendix G: Accuracy and precision for standards associated with data presented in Chapter 3, exclusive of those used to generate calibration curves.**

| | | | $\delta^{13}$C (‰, VPDB) | | $\delta^{15}$N (‰, AIR) | | C (wt. %) | | N (wt. %) | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Standard** | **RUN ID** | **n** | **Mean** | ***SD*** | **Mean** | ***SD*** | **Mean** | ***SD*** | **Mean** | ***SD*** |
| **Keratin** | **CN 14-06**[a] | **10** | –23.98 | 0.11 | | | | | | |
| | **CN 14-10**[b] | **9** | –23.99 | 0.05 | | | 47.54 | 0.21 | | |
| | **CN 14-11**[b] | **10** | –24.02 | 0.05 | | | 48.01 | 0.56 | | |
| | **CN 14-14**[a] | **9** | –24.11 | 0.05 | | | | | | |
| | **CN 14-17**[b] | **9** | –24.07 | 0.05 | | | 47.00 | 0.49 | | |
| | **CN 14-18**[b] | **8** | –24.08 | 0.04 | | | 48.05 | 0.48 | | |
| | **CN 14-21**[a] | **8** | –24.08 | 0.04 | | | | | | |
| | **CN 14-22** | **7** | –24.03 | 0.08 | | | 46.62 | 0.21 | | |
| | **CN 14-27** | **4** | –24.04 | 0.03 | | | 46.63 | 0.48 | | |
| | **N 14-01** | **9** | | | +6.35 | 0.11 | | | 14.56 | 0.23 |
| | **N 14-02** | **5** | | | +6.44 | 0.06 | | | 14.27 | 0.39 |
| | **N 14-03** | **4** | | | +6.37 | 0.13 | | | 14.54 | 0.18 |
| | **NO 14-08** | **15** | | | +6.49 | 0.12 | | | 14.31 | 0.38 |
| | **NO 14-10** | **7** | | | +6.40 | 0.20 | | | 14.37 | 0.36 |
| | **NO 14-11** | **15** | | | +6.42 | 0.11 | | | 14.34 | 0.21 |
| | **NO 14-12** | **15** | | | +6.41 | 0.12 | | | 14.04 | 0.23 |
| | **NO 14-14** | **16** | | | +6.42 | 0.10 | | | 13.76 | 0.39 |
| | **NO 14-15** | **13** | | | +6.46 | 0.10 | | | 14.28 | 0.42 |
| | **NO 14-21** | **14** | | | +6.44 | 0.09 | | | 14.08 | 0.65 |
| | **EA alone 14-13** | **2** | | | | | 48.96 | 0.71 | | |
| | **EA alone 14-14** | **3** | | | | | 49.38 | 0.27 | | |
| | **EA alone 14-15** | **6** | | | | | 49.09 | 0.82 | | |
| **IAEA-CH-6** | **CN 14-06** | **4** | –10.47 | 0.16 | | | | | | |
| | **CN 14-10** | **4** | –10.50 | 0.08 | | | | | | |
| | **CN 14-11** | **4** | –10.49 | 0.06 | | | | | | |
| | **CN 14-14** | **3** | –10.52 | 0.07 | | | | | | |
| | **CN 14-17** | **2** | –10.61 | 0.11 | | | | | | |
| | **CN 14-18** | **3** | –10.47 | 0.10 | | | | | | |

| | | | $\delta^{13}$C (‰, VPDB) | | $\delta^{15}$N (‰, AIR) | | C (wt. %) | | N (wt. %) | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Standard** | **SESSION ID** | **n** | **Mean** | ***SD*** | **Mean** | ***SD*** | **Mean** | ***SD*** | **Mean** | ***SD*** |
| | **CN 14-21** | **2** | −10.49 | 0.07 | | | | | | |
| | **CN 14-22** | **2** | −10.52 | 0.10 | | | | | | |
| | **CN 14-27** | **2** | −10.43 | 0.02 | | | | | | |
| **USGS40** | **CN 14-06** | **5** | | 0.14 | | | | | | |
| | **CN 14-10** | **5** | | 0.04 | | | | | | |
| | **CN 14-11** | **5** | | 0.06 | | | | | | |
| | **CN 14-14** | **4** | | 0.10 | | | | | | |
| | **CN 14-17** | **4** | | 0.02 | | | | | | |
| | **CN 14-18** | **4** | | 0.04 | | | | | | |
| | **CN 14-21** | **3** | | 0.05 | | | | | | |
| | **CN 14-22** | **2** | | 0.05 | | | | | | |
| | **CN 14-27** | **2** | | 0.02 | | | | | | |
| | **N 14-01** | **4** | | 0.02 | | | | | | |
| | **N 14-02** | **4** | | 0.30 | | | | | | |
| | **N 14-03** | **2** | | 0.01 | | | | | | |
| | **NO 14-08** | **5** | | 0.15 | | | | | | |
| | **NO 14-10** | **3** | | 0.09 | | | | | | |
| | **NO 14-11** | **5** | | 0.12 | | | | | | |
| | **NO 14-12** | **5** | | 0.08 | | | | | | |
| | **NO 14-14** | **5** | | 0.08 | | | | | | |
| | **NO 14-15** | **4** | | 0.19 | | | | | | |
| | **NO 14-21** | **5** | | 0.11 | | | | | | |
| **USGS41** | **CN 14-06** | **5** | | 0.16 | | | | | | |
| | **CN 14-10** | **5** | | 0.05 | | | | | | |
| | **CN 14-11** | **5** | | 0.05 | | | | | | |
| | **CN 14-14** | **4** | | 0.07 | | | | | | |
| | **CN 14-17** | **4** | | 0.09 | | | | | | |
| | **CN 14-18** | **5** | | 0.12 | | | | | | |
| | **CN 14-21** | **3** | | 0.06 | | | | | | |
| | **CN 14-22** | **3** | | 0.06 | | | | | | |

| | | | $\delta^{13}$C (‰, VPDB) | | $\delta^{15}$N (‰, AIR) | | C (wt. %) | | N (wt. %) | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Standard** | **SESSION ID** | **n** | **Mean** | ***SD*** | **Mean** | ***SD*** | **Mean** | ***SD*** | **Mean** | ***SD*** |
| | **CN 14-27** | **2** | | 0.03 | | | | | | |
| | **N 14-01** | **4** | | 0.10 | | | | | | |
| | **N 14-02** | **5** | | 0.14 | | | | | | |
| | **N 14-03** | **2** | | 0.02 | | | | | | |
| | **NO 14-08** | **5** | | 0.13 | | | | | | |
| | **NO 14-10** | **3** | | 0.04 | | | | | | |
| | **NO 14-11** | **4** | | 0.10 | | | | | | |
| | **NO 14-12** | **4** | | 0.06 | | | | | | |
| | **NO 14-14** | **5** | | 0.15 | | | | | | |
| | **NO 14-15** | **3** | | 0.19 | | | | | | |
| | **NO 14-21** | **4** | | 0.17 | | | | | | |
| **NIST 1547** | **NO 14-08** | **9** | | | | | | | 2.77 | 0.06 |
| | **NO 14-10** | **5** | | | | | | | 2.76 | 0.02 |
| | **NO 14-11** | **8** | | | | | | | 2.57 | 0.02 |
| | **NO 14-12** | **8** | | | | | | | 2.76 | 0.02 |
| | **NO 14-14** | **9** | | | | | | | 2.63 | 0.09 |
| | **NO 14-15** | **4** | | | | | | | 2.69 | 0.06 |
| | **NO 14-21** | **7** | | | | | | | 2.67 | 0.04 |
| **Acetanilide** | **[c]1108 EA 14-13** | **8** | | | | | | 1.10 | | 0.11 |
| | **1108 EA 14-14** | **9** | | | | | | 0.73 | | 0.11 |
| | **1108 EA 14-15** | **9** | | | | | | 0.66 | | 0.10 |
| **High OC** | **1108 EA 13-05 (Soil)** | **6** | | | | | | 0.06 | | |
| | **1108 EA 14-01 (Soil)** | **6** | | | | | | | | 0.01 |
| **Low OC** | **1108 EA 13-05 (Soil)** | **6** | | | | | | 0.01 | | |
| | **1108 EA 14-01 (Soil)** | **6** | | | | | | | | 0.01 |
| **NBS-19** | **MPRP 15-01** | **5** | | 0.05 | | | | | | |

| | | | $\delta^{13}$C (‰, VPDB) | | $\delta^{15}$N (‰, AIR) | | C (wt. %) | | N (wt. %) | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Standard** | **SESSION ID** | **n** | **Mean** | ***SD*** | **Mean** | ***SD*** | **Mean** | ***SD*** | **Mean** | ***SD*** |
| **LSEVC** | **MPRP 15-01** | **3** | | 0.29 | | | | | | |
| **WS-1** | **MPRP 15-01** | **3** | +0.69 | 0.11 | | | | | | |
| **Suprapur** | **MPRP 15-01** | **2** | −35.78 | 0.01 | | | | | | |

| | | | $\delta^{18}$O (‰, VPDB) | | $\delta^{2}$H (‰, AIR) | |
|---|---|---|---|---|---|---|
| **Standard** | **SESSION ID** | **n** | **Mean** | ***SD*** | **Mean** | ***SD*** |
| **NBS-19** | **MPRP 15-01** | **6** | | 0.09 | | |
| **NBS-18** | **MPRP 15-01** | **3** | | 0.13 | | |
| **Suprapur** | **MPRP 15-01** | **2** | +13.28 | 0.08 | | |
| **LSD** | **GBWO 15-01** | **2** | −22.57 | 0.00 | | |
| | **GBWH 14-01** | **3** | | | −161.80 | 3.96 |

| | | | $\delta^{18}$O (‰, VPDB) | | $\delta^{2}$H (‰, AIR) | |
|---|---|---|---|---|---|---|
| **Standard** | **SESSION ID** | **n** | **Mean** | ***SD*** | **Mean** | ***SD*** |
| **MID** | **GBWO 15-01** | **2** | −13.03 | 0.05 | | |
| | **GBWH 14-01** | **3** | | | −107.37 | 1.93 |
| **EDT** | **GBWO 15-01** | **2** | −7.22 | 0.10 | | |
| | **GBWH 14-01** | **4** | | | −54.96 | 1.04 |
| **Heaven** | **GBWO 15-01** | **2** | −0.27 | 0.02 | | |
| | **GBWH 14-01** | **3** | | | +88.70 | 2.77 |

**[a] The entire analytical session was repeated using the Fisons 1108 Elemental Analyzer for elemental composition.**
**[b] Elemental data were corrected, as described in Appendix B.**
**[c] 1108 EA: Fisons 1108 Elemental Analyzer**

**Appendix H: (H.1) $\delta^{13}C$ and $\delta^{15}N$ of all replicates in the decomposition experiment.**

| | $\delta^{13}C$ (‰, VPDB) | | | | | | $\delta^{15}N$ (‰, AIR) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Buried | | | Not buried | | | Buried | | | Not buried | | |
| | Time 1 | | | | | | | | | | | |
| **Plant ID** | **R1** | **R2** | **R3** | **R1** | **R2** | **R3** | **R1** | **R2** | **R3** | **R1** | **R2** | **R3** |
| ***E. trachycaulus*** | −27.7 | −27.7 | −27.8 | −27.7 | −27.7 | −27.8 | +1.0 | −2.1 | −1.8 | +1.0 | −2.1 | −1.8 |
| ***C. purpurascens*** | −27.3 | −27.6 | −27.5 | −27.3 | −27.6 | −27.5 | −0.3 | −0.9 | −1.2 | −0.3 | −0.9 | −1.2 |
| ***P. glauca*** | −26.4 | −25.6 | −24.9 | −26.4 | −25.7 | −24.9 | +0.6 | 0.5 | +0.8 | +0.6 | +0.5 | +0.8 |
| ***F. altaica*** | −27.6 | −27.6 | −27.6 | −27.6 | −27.6 | −27.6 | −0.8 | −6.3 | −7.0 | −0.8 | −6.3 | −7.0 |
| ***A. frigida*** | −30.1 | −29.0 | −29.3 | −30.1 | −29.0 | −29.3 | −3.6 | −1.3 | −2.4 | −3.6 | −1.3 | −2.4 |
| ***E. spicatus*** | −27.0 | −27.4 | −26.9 | −27.0 | −27.4 | −26.9 | −1.9 | −2.8 | −4.6 | −1.9 | −2.8 | −4.6 |
| | Time 2 | | | | | | | | | | | |
| ***E. trachycaulus*** | −28.1 | −26.7 | −27.6 | −28.3 | −28.7 | −28.2 | +6.8 | +3.1 | +5.8 | −0.8 | −1.0 | +1.2 |
| ***C. purpurascens*** | −24.5 | −24.4 | −24.3 | −25.0 | −25.1 | −24.6 | +3.6 | +2.4 | +3.5 | −1.9 | −1.6 | −0.3 |
| ***P. glauca*** | −26.1 | −26.7 | −26.3 | −26.4 | −26.8 | −26.8 | 0.0 | +1.7 | 2.0 | −1.0 | −1.3 | −0.5 |
| ***F. altaica*** | −27.1 | −27.5 | −27.2 | −28.0 | −27.5 | −27.6 | +5.2 | +0.5 | 5.3 | −1.7 | +0.2 | +1.0 |
| ***A. frigida*** | −30.8 | −30.5 | −30.8 | −29.3 | −29.6 | −29.3 | −0.5 | −1.1 | −0.8 | −2.2 | −2.0 | −1.7 |
| ***E. spicatus*** | −27.8 | −27.8 | −26.6 | −27.2 | −26.9 | −27.1 | +4.2 | +4.0 | +3.6 | −3.0 | −2.4 | −0.2 |

**App. H.1. Cont'd.**

| | $\delta^{13}$C (‰, VPDB) | | | | | | $\delta^{15}$N (‰, AIR) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Buried | | | Not buried | | | Buried | | | Not buried | | |
| | Time 3 | | | | | | | | | | | |
| **Plant ID** | **R1** | **R2** | **R3** | **R1** | **R2** | **R3** | **R1** | **R2** | **R3** | **R1** | **R2** | **R3** |
| ***E. trachycaulus*** | −29.2 | −28.0 | −28.7 | −27.5 | −28.4 | −27.0 | +3.9 | +5.1 | +4.4 | −1.8 | −0.8 | −0.8 |
| ***C. purpurascens*** | −25.4 | −23.8 | −24.7 | −25.6 | −24.8 | −24.9 | +3.4 | +2.4 | +3.0 | +0.7 | −5.0 | +0.2 |
| ***P. glauca*** | −26.4 | −27.1 | −26.7 | −26.4 | −27.5 | −26.8 | +2.1 | +3.7 | +2.1 | −3.4 | −2.6 | −1.5 |
| ***F. altaica*** | −28.2 | −28.0 | −27.7 | −27.2 | −27.6 | −27.5 | +6.8 | +7.1 | +5.5 | −4.2 | −0.1 | +1.3 |
| ***A. frigida*** | −29.2 | −30.5 | −30.9 | −29.6 | −29.5 | −30.0 | +0.7 | +1.3 | +0.8 | +1.9 | +0.3 | −1.8 |
| ***P. spicata*** | −28.4 | −28.1 | −25.4 | −27.2 | −26.8 | −28.7 | +0.5 | +6.1 | +6.3 | −5.2 | −2.7 | −2.2 |
| | Time 4 | | | | | | | | | | | |
| ***E. trachycaulus*** | −28.7 | −28.1 | −27.0 | −27.9 | −27.0 | −27.4 | +2.8 | +3.3 | +0.9 | −3.8 | −3.2 | −1.6 |
| ***C. purpurascens*** | −25.8 | −26.1 | −24.7 | −24.0 | −25.9 | −24.5 | +2.2 | +3.1 | +3.4 | −2.3 | −2.0 | −0.7 |
| ***P. glauca*** | −27.9 | −27.0 | −26.8 | −27.1 | −27.2 | −27.0 | +3.0 | +3.1 | +3.1 | −3.8 | −2.6 | −2.9 |
| ***F. altaica*** | −27.6 | −26.6 | −28.1 | −25.4 | −27.3 | −27.3 | +5.0 | +5.6 | +5.1 | −1.6 | −1.6 | −1.8 |
| ***A. frigida*** | −30.6 | −31.1 | −30.4 | −29.4 | −29.5 | −28.8 | −0.1 | +0.5 | +0.3 | −1.3 | −1.5 | −0.9 |
| ***E. spicatus*** | −27.3 | −26.8 | −27.6 | −27.5 | −28.0 | −26.9 | +3.7 | +4.2 | +4.4 | −5.4 | −2.9 | −4.3 |

**R: Replicate**

**Appendix H.2: C and N contents of all replicates in the decomposition experiment.**

| | C (wt. %) | | | | | | N (wt. %) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Buried | | | Not buried | | | Buried | | | Not buried | | |
| | Time 1 | | | | | | | | | | | |
| **Plant ID** | **R1** | **R2** | **R3** | **R1** | **R2** | **R3** | **R1** | **R2** | **R3** | **R1** | **R2** | **R3** |
| ***E. trachycaulus*** | 42.9 | 41.9 | 44.3 | 42.9 | 41.9 | 44.3 | 0.3 | 1.0 | 0.8 | 0.3 | 1.0 | 0.8 |
| ***C. purpurascens*** | 39.1 | 41.0 | 43.0 | 39.1 | 41.0 | 43.0 | 0.6 | 0.7 | 0.5 | 0.6 | 0.7 | 0.5 |
| ***P. glauca*** | 41.0 | 41.4 | 40.6 | 41.0 | 41.4 | 40.6 | 0.9 | 1.5 | 1.3 | 0.9 | 1.5 | 1.3 |
| ***F. altaica*** | 39.1 | 42.6 | 41.2 | 39.1 | 42.6 | 41.2 | 0.3 | 0.9 | 0.9 | 0.3 | 0.9 | 0.9 |
| ***A. frigida*** | 44.0 | 45.9 | 44.8 | 44.0 | 45.9 | 44.8 | 1.3 | 1.4 | 1.5 | 1.3 | 1.4 | 1.5 |
| ***E. spicatus*** | 42.4 | 41.7 | 44.9 | 42.4 | 41.7 | 44.9 | 0.5 | 0.8 | 0.2 | 0.5 | 0.8 | 0.2 |
| | Time 2 | | | | | | | | | | | |
| ***E. trachycaulus*** | 43.0 | 39.0 | 40.6 | 40.6 | 43.2 | 40.4 | 0.5 | 0.6 | 1.1 | 0.4 | 0.4 | 1.1 |
| ***C. purpurascens*** | 40.8 | 40.0 | 37.0 | 39.9 | 37.5 | 37.9 | 0.7 | 0.8 | 0.8 | 0.5 | 0.6 | 0.6 |
| ***P. glauca*** | 40.2 | 38.9 | 40.6 | 42.3 | 39.4 | 40.0 | 1.0 | 0.9 | 0.8 | 0.7 | 0.9 | 1.1 |
| ***F. altaica*** | 28.2 | 38.2 | 34.3 | 37.8 | 34.1 | 32.1 | 0.8 | 0.5 | 0.7 | 0.4 | 0.6 | 0.7 |
| ***A. frigida*** | 48.2 | 46.5 | 46.9 | 48.9 | 45.2 | 44.7 | 1.1 | 1.1 | 0.9 | 1.2 | 1.3 | 1.2 |
| ***E. spicatus*** | 42.7 | 39.2 | 41.9 | 39.3 | 41.4 | 39.9 | 0.7 | 0.9 | 0.9 | 0.6 | 0.6 | 0.7 |

**App. H.2. Cont'd.**

| | C (wt. %) | | | | | | N (wt. %) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Buried | | | Not buried | | | Buried | | | Not buried | | |
| | Time 3 | | | | | | | | | | | |
| **Plant ID** | **R1** | **R2** | **R3** | **R1** | **R2** | **R3** | **R1** | **R2** | **R3** | **R1** | **R2** | **R3** |
| ***E. trachycaulus*** | 42.1 | 31.5 | 38.9 | 41.8 | 44.5 | 32.4 | 1.3 | 1.3 | 1.2 | 0.5 | 0.6 | 1.0 |
| ***C. purpurascens*** | 37.4 | 34.6 | 30.1 | 34.7 | 43.8 | 35.6 | 1.3 | 0.9 | 1.5 | 0.9 | 0.4 | 0.7 |
| ***P. glauca*** | 24.6 | 30.5 | 29.4 | 38.8 | 35.9 | 28.6 | 1.2 | 1.5 | 1.5 | 0.8 | 0.8 | 1.0 |
| ***F. altaica*** | 35.8 | 28.0 | 31.6 | 42.4 | 36.0 | 24.6 | 1.7 | 1.3 | 1.0 | 0.3 | 0.6 | 0.8 |
| ***A. frigida*** | 40.8 | 44.0 | 42.8 | 46.3 | 49.6 | 42.5 | 2.2 | 1.5 | 1.6 | 1.2 | 1.0 | 1.1 |
| ***E. spicatus*** | 34.2 | 40.8 | 31.6 | 40.4 | 40.0 | 37.0 | 0.8 | 0.5 | 1.4 | 0.4 | 0.5 | 0.6 |
| | Time 4 | | | | | | | | | | | |
| ***E. trachycaulus*** | 41.9 | 35.9 | 35.7 | 38.8 | 37.3 | 39.2 | 1.2 | 1.4 | 1.6 | 0.5 | 0.5 | 0.6 |
| ***C. purpurascens*** | 31.1 | 39.4 | 38.0 | 34.1 | 29.3 | 34.7 | 1.2 | 1.1 | 1.2 | 0.7 | 0.9 | 0.8 |
| ***P. glauca*** | 29.1 | 30.3 | 28.5 | 29.5 | 30.9 | 32.2 | 1.5 | 1.6 | 1.5 | 0.9 | 1.0 | 0.9 |
| ***F. altaica*** | 36.0 | 31.0 | 29.6 | 24.1 | 28.4 | 38.5 | 1.1 | 1.0 | 1.3 | 0.7 | 0.7 | 0.5 |
| ***A. frigida*** | 45.8 | 43.1 | 39.1 | 45.6 | 44.8 | 43.0 | 1.1 | 1.4 | 1.3 | 1.3 | 1.2 | 1.2 |
| ***E. spicatus*** | 29.3 | 23.2 | 25.8 | 39.1 | 36.4 | 37.0 | 1.2 | 1.0 | 1.1 | 0.5 | 0.7 | 0.6 |

**R: Replicate**

**Appendix I:**

# Effects of Grazing and Dung Fertilization on Plant Nitrogen Isotopic Compositions: Controlled Experiments

## Introduction

Megaherbivore activities, including grazing, trampling and excretory (urine and dung) fertilization, can introduce more labile forms of nitrogen and subsequently change its dynamics in soils and plants (Frank et al., 2000; McNaughton et al., 1988; Semmartin et al., 2004; Zheng et al., 2012). Given that N availability is one of the important factors limiting the productivity of most terrestrial ecosystems (Lebauer and Treseder, 2008), herbivore effects have been studied for many different ecosystems (Coetsee et al., 2010; Frank and Evans, 1997; Li et al., 2010; McKendrick et al., 1980; Ruess and McNaughton, 1987). Many studies have suggested a key role for megaherbivores in sustaining the productivity of late Pleistocene ecosystems, mainly through their effects on nutrients dynamics – particularly N (Daufresne, 2013; Doughty et al., 2013; Gill, 2013; Tanentzap et al., 2013). Chapter 3 of this dissertation suggests that the N cycle in eastern Beringia was more open during the late Pleistocene than at the present time. The potential role of herbivory (grazing and dung fertilization) in causing such a change in N cycling in Beringia is therefore worthy of examination.

Accordingly, the purpose of this study is to assess the N isotopic responses of two of the most common subarctic, perennial grass species, *Poa glauca* and *Elymus macrourus* (Swanson, 2006; Zazula et al., 2007), to herbivory (grazing and dung fertilization). These plants were grown from seed in controlled-growth chambers under three different levels of cutting and fertilization intensities and two tissues (leaf and fine root) were subsequently analyzed for C and N isotopic compositions.

# Materials and Methods

## Growth-Chamber Conditions and Design of Experiments

*P. glauca* and *E. macrourus* were initially grown from seed in small pots (13 cm sides × 14 cm height, ~2.4 L) filled with loess soil collected from the eastern shoreline of Kluane Lake. Seed was provided by Randy Lewis (Arctic Alpine Reclamation group). All conditions (watering interval, temperature (T), relative humidity (RH), photosynthetic photon flux density (PPFD) and photoperiod (PP)) provided for these plants during germination and growth in controlled-growth chambers were similar to those described for the $CO_2$ experiments (section 4.2.1). $CO_2$ concentrations were maintained at ambient levels (411 ± 20 $\mu$mol C mol$^{-1}$) and monitored continuously using a computer-controlled, $CO_2$ infra-red gas analyzer (Model: WMA-4 $CO_2$ Analyzer, PP Systems International, Inc., Amesbury, MA, USA). This experiment was conducted only for plants grown from seeds in ~2.4 L pots (see section 4.2.1) for 60 days after the seedlings were transported to the growth-chambers. Each experiment (Grazing and Fertilization) was conducted in separate controlled-growth chambers at three different levels of grazing intensity and N fertilization, as discussed below.

The 'Grazing Experiment' involved: (i) no cutting (T0), (ii) cutting every 10 and 16 days for *E. macrourus* and *P. glauca*, respectively (T1), and cutting every 20 and 26 days for *E. macrourus* and *P. glauca*, respectively (T2). Five replicates were made for each species for each treatment. The cutting frequency chosen for species depended on their growth rate, which was higher for *E. macrourus* than *P. glauca*. Cuttings were performed using scissor to a height of ~1 cm above ground, thus maintaining the collar region that contains meristems (Chapman and Lemaire, 1993) needed to regrow the plants after each defoliation.

The 'Fertilization Experiment' utilized composted cow manure (N = 2 wt. %, and $\delta^{15}$N = 1.5 ‰). The fertilization levels were: (i) none (T0), (ii) 0.5 g N/L (T1) and (iii) 1 g N/L (T2). Prior to use, the cow manure was prepared as described in Chapter 4 (section 4.2.1).

The cow manure was mixed completely into whole soils in order to limit N loss through volatilization. Five replicates were made for each species for each treatment. At the end of 60 days two different plant parts (leaves and fine roots) were sampled for N and C isotopic analysis.

### Sample Preparation and Isotopic Analysis

Plant samples were prepared for isotopic analysis following the procedures explained in Chapter 2 (section 2.2.1), and their C and N isotopic compositions were measured following the protocol described in Chapter 2 (section 2.2.3). Accuracy and precision were monitored using the laboratory keratin and IAEA-CH-6 (sucrose) standards. The average $\delta^{13}C$ obtained for keratin was –24.07 ± 0.06 ‰ (n = 33), which compares well with its accepted value of –24.05 ± 0.15 ‰. The average $\delta^{13}C$ obtained for IAEA-CH-6 was –10.53 ± 0.10 ‰ (n = 10), which compares well with its accepted value –10.45 ± 0.03 ‰ (Coplen et al., 2006). Sample reproducibility for duplicates was ± 0.06 ‰ for $\delta^{13}C$ (n = 12). The average $\delta^{15}N$ of keratin was +6.42 ± 0.12 ‰ (n = 41), which compares well with its accepted value of +6.36 ‰. Sample reproducibility for duplicates was ± 0.09 ‰ for $\delta^{15}N$ (n = 15). Analytical accuracy and precision for all standards associated with data presented here are listed in Appendix J.

## Results and Discussion

Table I-1 and I-2 present the C and N isotopic compositions of fine root and leaf obtained in the grazing and fertilization experiments.

**Table I-1: *P. glauca* $\delta^{13}$C and $\delta^{15}$N for Grazing and Fertilization Experiments.**

| | | | $\delta^{13}$C (‰, VPDB) | | | | | | $\delta^{15}$N (‰, AIR) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Grazing | | | Fertilization | | | Grazing | | | Fertilization | | |
| | | | Treatments | | | | | | | | | | | |
| **Plant ID** | **Tissue** | **Re** | **T0** | **T1** | **T2** | **T0** | **T1** | **T2** | **T0** | **T1** | **T2** | **T0** | **T1** | **T2** |
| ***P. glauca*** | **Fine Root** | **1** | −26.7 | **−26.4** | −26.3 | −25.7 | −25.6 | −26.2 | −1.2 | **−0.5** | −2.9 | +1.5 | +0.4 | +0.8 |
| | | **2** | −26.4 | −25.7 | −26.1 | −25.7 | −24.9 | −24.3 | −0.1 | −0.3 | −1.5 | +0.5 | −1.6 | −1.6 |
| | | **3** | −25.8 | −26.3 | −26.3 | −25.8 | −25.0 | NA | −1.6 | −1.3 | −2.7 | +0.9 | 0.2 | NA |
| | | **4** | −25.8 | −26.3 | −26.2 | −26.0 | −25.6 | −26.5 | −1.5 | −1.5 | −0.2 | +0.4 | +1.6 | 0.3 |
| | | **5** | −25.5 | −25.9 | −25.9 | −25.9 | −24.8 | **−26.1** | 0.4 | −1.4 | −2.2 | +1.1 | +0.3 | −1.3 |
| | **Leaf** | **1** | −27.2 | −26.7 | −26.4 | −27.2 | −26.3 | −28.1 | −1.5 | −2.0 | −1.8 | −0.9 | 0.0 | −0.8 |
| | | **2** | −27.3 | −26.2 | −26.0 | −27.1 | **−25.9** | −25.0 | −0.6 | −2.1 | −2.0 | −1.0 | 0.8 | +0.6 |
| | | **3** | −26.4 | −26.1 | −27.0 | −26.3 | −24.5 | NA | −2.0 | −1.7 | −2.6 | −1.4 | −0.2 | – |
| | | **4** | −26.0 | −26.8 | **−27.0** | −26.5 | −27.3 | −27.6 | −1.5 | −1.3 | −1.7 | −1.0 | −0.4 | −1.0 |
| | | **5** | −27.3 | −27.0 | −26.6 | −26.7 | −24.6 | −26.5 | −0.7 | −2.1 | −0.9 | −2.1 | **+1.2** | −2.2 |

**Grazing T0:** No cutting**, T1:** Cutting every 16 days**, T2: C**utting every 26 days.
**Fertilization T0:** 0 gr N/L**, T1:** 0.5 gr N/L**, T2:**1 gr N/L.
**Re: Replicate.**
**NA: data not available.**
**Boldface denotes average of duplicate analyses.**

**Table I-2: *E. macrourus* $\delta^{13}$C and $\delta^{15}$N for Grazing and Fertilization Experiments.**

| | | | $\delta^{13}$C (‰, VPDB) | | | | | | $\delta^{15}$N (‰, AIR) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Grazing | | | Fertilization | | | Grazing | | | Fertilization | | |
| | | | Treatments | | | | | | | | | | | |
| **Plant ID** | **Tissue** | **Re** | **T0** | **T1** | **T2** | **T0** | **T1** | **T2** | **T0** | **T1** | **T2** | **T0** | **T1** | **T2** |
| ***E. macrourus*** | **Fine Root** | **1** | −28.0 | −28.0 | −28.0 | −27.1 | −27.9 | −27.3 | −0.2 | **−2.1** | −0.9 | +0.2 | −1.0 | −0.6 |
| | | **2** | −28.5 | −27.9 | **−27.4** | −27.9 | −27.1 | −26.9 | 0.6 | −2.1 | −2.0 | −2.0 | −2.2 | **+0.7** |
| | | **3** | −28.5 | −28.2 | **−28.8** | −28.0 | −27.4 | −27.0 | −0.1 | −1.2 | **−1.1** | −0.4 | −2.2 | **−1.4** |
| | | **4** | −28.3 | −27.4 | −28.1 | −27.8 | −28.1 | −26.8 | −0.3 | −0.9 | −1.3 | −1.3 | 1.0 | +0.8 |
| | | **5** | −28.0 | −27.8 | −28.2 | −28.5 | −27.9 | **−26.9** | −1.3 | −0.9 | −0.6 | −1.1 | 0.4 | **+0.6** |
| | **Leaf** | **1** | −29.1 | −27.3 | −28.3 | **−28.9** | −29.5 | −28.1 | +2.1 | +0.5 | **+1.9** | **−0.1** | 1.4 | +0.3 |
| | | **2** | −28.9 | −28.2 | −27.4 | −29.3 | −28.8 | −28.0 | +2.7 | +1.0 | +0.1 | −0.1 | 0.4 | +0.7 |
| | | **3** | −29.2 | −28.2 | −28.9 | −29.7 | −28.6 | −27.8 | +2.4 | +1.4 | +2.5 | +0.1 | +0.1 | +0.4 |
| | | **4** | −27.8 | −27.4 | −28.6 | −29.3 | −30.3 | −28.7 | +2.0 | +1.4 | +1.4 | −0.2 | +2.2 | +1.1 |
| | | **5** | **−27.8** | −28.0 | **−28.7** | −29.9 | −29.7 | −28.0 | +1.7 | +1.7 | +1.3 | +0.5 | +1.9 | +0.7 |

**Grazing T0:** No cutting**, T1:** Cutting every 10 days**, T2: C**utting every 20 days.
**Fertilization T0:** 0 gr N/L**, T1:** 0.5 gr N/L**, T2:**1 gr N/L.
**Re: Replicate.**
**NA: data not available.**
**Boldface denotes average of duplicate analyses.**

## $\delta^{13}C$ Variations in Grazing Experiment

Table I-3 presents the average isotopic differences between fine root and leaf ($\Delta^{13}C_{FR-L}$) for each individual plant for each treatment for both species examined in this experiment. In all three treatments (T0, T1 and T2) and for both species, $\Delta^{13}C_{FR-L}$ is positive and leaves have more negative $\delta^{13}C$ than fine roots. This result matches that measured for plants in the $CO_2$ experiment (Chapter 4) and naturally occurring plants (Chapter 2), and is in agreement with many other studies of $C_3$ plants (Brugnoli and Farquhar, 2000; Cernusak et al., 2009; Hobbie and Werner, 2004; Scartazza et al., 1998).

From experiment T0 to T1, a slight, non-significant increase in $\delta^{13}C$ of both tissues is observed, except for fine root of *P. glauca* (Figs. I-1-1, I-1-2). In T2 (higher cutting intensity), both plant tissues in both species have lower $\delta^{13}C$ than in T1 and are closer to values measured for T0. None of these changes, however, are statistically significant. In general, no significant shift in plant $\delta^{13}C$ is observed with the change in cutting intensity.

## $\delta^{13}C$ Variations in Fertilization Experiment

In the fertilization experiment, $\Delta^{13}C_{FR-L}$ is positive for both species and on average leaves have more negative $\delta^{13}C$ than fine roots (Table I-3) for all three treatments (T0, T1 and T2) (Fig. I-2). With the application of N fertilizer in T1, both leaves and fine roots in *P. glauca* show a non-significant increase in $\delta^{13}C$ (Fig. I-2-1) relative to T0, while the $\delta^{13}C$ of these tissues in *E. macrourus* does not change much from T0 (Fig. I-2-2). The $\delta^{13}C$ of *P. glauca* decreased in T2 relative to T1 (Fig. I-2-1) but the change is not significant. For *E. macrourus* in T2, the increased amount of N fertilizer is associated with higher $\delta^{13}C$ for both leaf ($p$-value$_{(T0\ vs.\ T2)}$= 0.003; $p$-value$_{(T1\ vs.\ T2)}$= 0.004) and fine root ($p$-value$_{(T0\ vs.\ T2)}$= 0.011; $p$-value$_{(T1\ vs.\ T2)}$= 0.044) than T0 and T1.

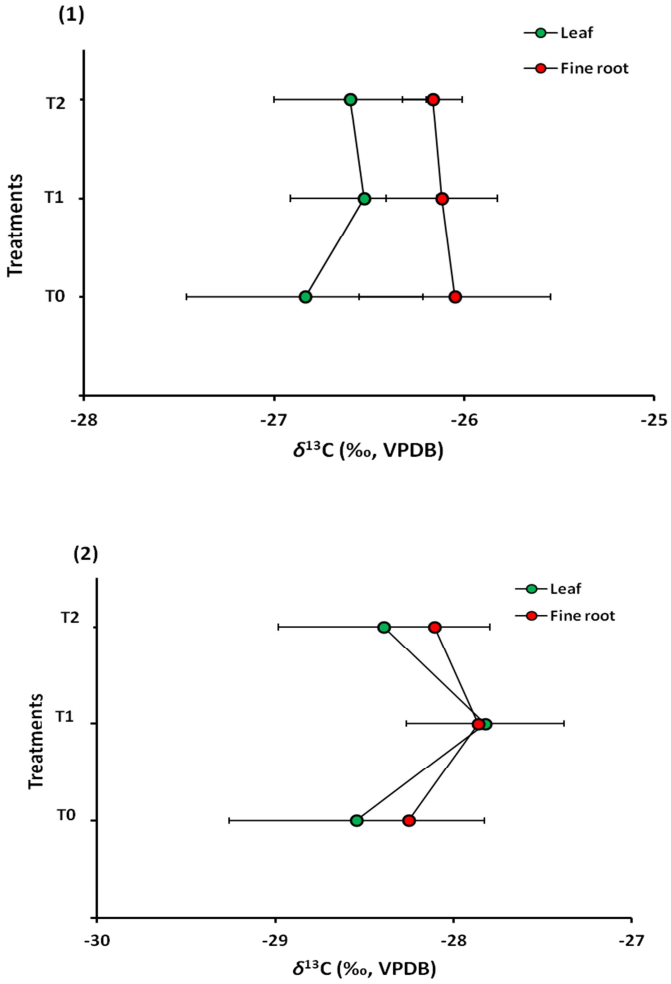


**Figure I-1: Change in $\delta^{13}$C of leaf and fine root with cutting intensity (T0, T1 and T2) for: (1) *P. glauca* and (2) *E. macrourus*. (Average ± SD (n = 5) are illustrated).**

Previous studies have related the influence of N fertilization on plant $\delta^{13}$C to plant water status (Clay et al., 2001; Jenkinson et al., 1995) and water use efficiency (Yin and Raven, 1998). Some suggest that a significant impact of N fertilization on plant $\delta^{13}$C is not expected (Szpak et al., 2012) but other studies report an increase (Iqbal et al., 2005; Jenkinson et al., 1995; Serret et al., 2008) and still others a decrease (Shangguan et al., 2000). The significant increase of $\delta^{13}$C in *E. macrourus* with fertilization in T2 may be related to some physiological response, such as a change in water use efficiency or stomatal conductance in response to greater N availability, and may be genotype dependent (Condon and Richards, 1993).

**Table I-3: Average $\Delta^{13}C_{FR-L}$ and $\Delta^{15}N_{FR-L}$ for Grazing and Fertilization Experiments.**

| Treatment | Plant Species | $\Delta^{13}C_{FR-L}$ (‰, VPDB) | $\Delta^{15}N_{FR-L}$ (‰, VPDB) |
|---|---|---|---|
| **Grazing Experiment** | | | |
| **T0** | ***P. glauca*** | +0.8 (± 0.6)* | +0.5 (± 0.4) |
| | ***E. macrourus*** | +0.7 (± 0.7) | −2.5 (± 0.3) |
| **T1** | ***P. glauca*** | +0.4 (± 0.5) | +0.8 (± 0.8) |
| | ***E. macrourus*** | 0.0 (± 0.4) | −2.7 (± 0.3) |
| **T2** | ***P. glauca*** | +0.4 (± 0.4) | −0.1 (± 1.2) |
| | ***E. macrourus*** | +0.3 (± 0.2) | −2.6 (± 0.7) |
| **Fertilization Experiment** | | | |
| **T0** | ***P. glauca*** | +0.9 (± 0.5) | +2.2 (± 0.7) |
| | ***E. macrourus*** | +1.6 (± 0.2) | −1.0 (± 0.9) |
| **T1** | ***P. glauca*** | +0.5 (± 0.9) | −0.1 (± 1.6) |
| | ***E. macrourus*** | +1.7 (± 0.4) | −2.0 (± 0.6) |
| **T2** | ***P. glauca*** | +1.1 (± 0.7) | +0.4 (± 1.8) |
| | ***E. macrourus*** | +1.1 (± 0.5) | −0.6 (± 0.7) |

***Values in parentheses are SD (n = 5).**

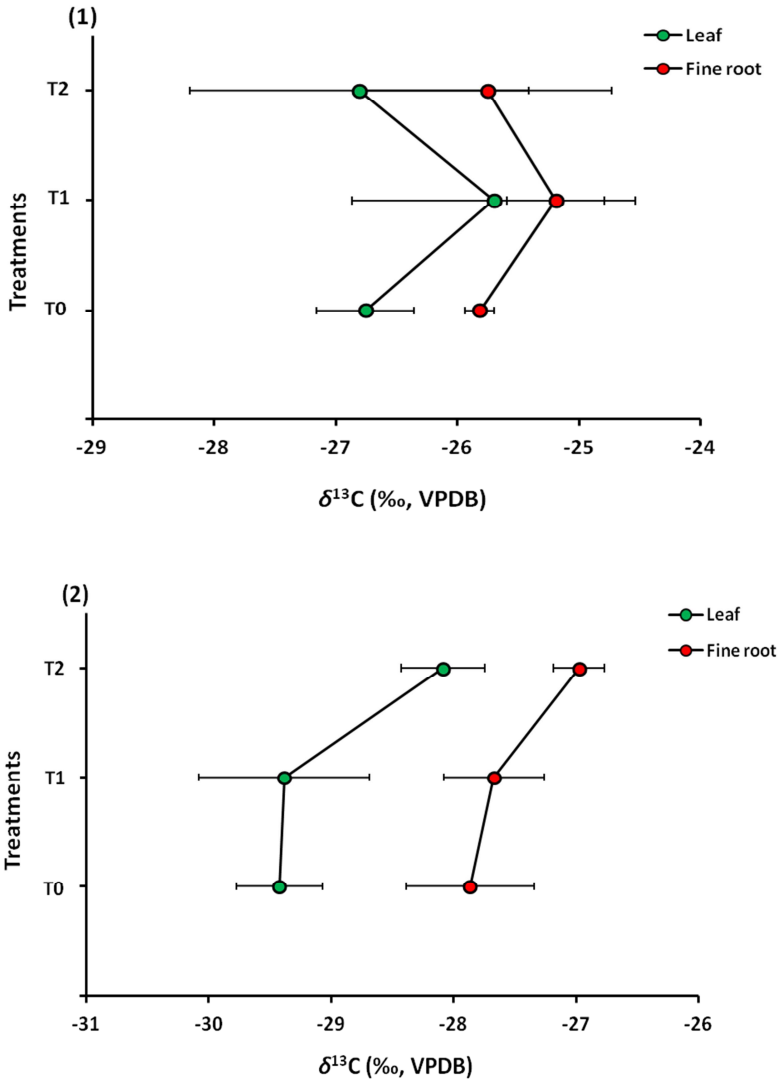


**Figure I-2: Change in $\delta^{13}$C of leaf and fine root with different fertilization intensity (T0, T1 and T2) for: (1) *P. glauca* and (2) *E. macrourus*. (Averages ± SD (n = 5) are illustrated).**

## $\delta^{15}$N Variations in Grazing Experiment

For *P. glauca*, fine roots on average have higher $\delta^{15}$N than leaves (Table I-3), while this pattern is reversed for *E. macrourus* (Table I-3). This observation for *E. macrourus* matches those previously reported for other plants in hydroponic and controlled growth experiments (Evans et al., 1996; Evans, 2001; Yoneyama and Kaneko, 1989). The

possible mechanisms responsible for such intra-plant variations have been discussed in Chapter 4 (section 4.4.1).

The opposite pattern observed for *P. glauca*, however, is not unexpected considering the possible different modes of N assimilation in different species (Evans, 2001). *P. glauca* might have lower capacity for nitrate reduction in roots than leaves. This could be a result of lower quantities of nitrate reductase in roots than leaves (Kolb and Evans, 2002) or a difference in nitrate reductase's sensitivity to amino acids produced during N assimilation, which could affect its activity (Guerrero et al., 1981; Kolb and Evans, 2002). Kolb et al. (2002) also have reported such a pattern for two species of deciduous trees.

In *Poa glauca*, with increase in cutting intensity from T0 to T2, a gradual, but not significant, reduction in $\delta^{15}$N in both fine roots and leaves is observed (Fig I-3-1). In *Elymus macrourus*, this pattern is the same from T0 to T1, but changes toward slightly more positive values from T1 to T2 (Fig I-3-2). None of these N isotopic shifts are statistically significant.

## $\delta^{15}$N Variations in Fertilization Experiment

The $\delta^{15}$N intra-plant variations between fine root and leaf observed for both *P. glauca* and *E. macrourus* during the fertilization experiment is similar to that observed in the grazing experiment (Table I-3). For *P. glauca*, most fine roots have higher $\delta^{15}$N than leaves which was discussed in previous section, while the pattern is opposite for *E. macrourus*, with leaves show higher $\delta^{15}$N than fine roots (Table I-3). Possible reasons for this behavior also have been discussed previously in Chapter 4 (section 4.4.1).

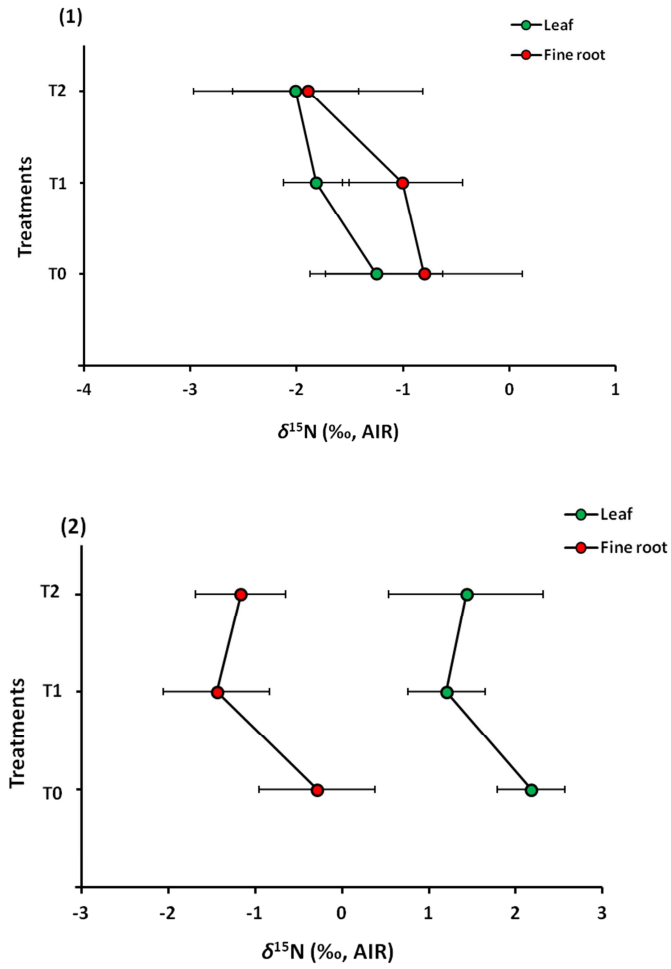


**Figure I-3: Change in $\delta^{15}$N with grazing intensity (T0, T1 and T2) for: (1) *P. glauca* and (2) *E. macrourus*. (Averages ± SD (n = 5) are illustrated).**

No specific pattern of change in $\delta^{15}$N of fine root is observed with increasing quantities of N fertilizer for *P. glauca* or *E. macrourus* (Fig. I-4-1, I-4-2)). For leaves, a positive shift from T0 to T1 and then a negative shift from T1 to T2 are observed for both species. The change in $\delta^{15}$N is statistically significant between T0 and T1 for *P. glauca* (*p*-value = 0.025) and between T0 and T2 for *E. macrourus* (*p*-value = 0.034).

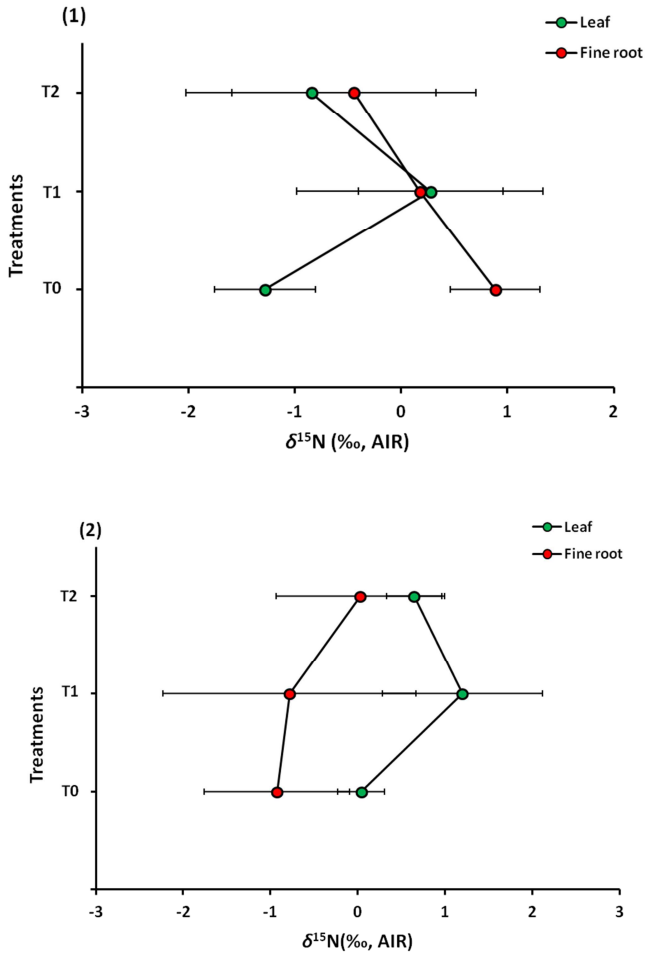


**Figure I-4: Change in $\delta^{15}$N with fertilization intensity (T0, T1 and T2) for: (1) *P. glauca* and (2) *E .macrourus*. (Averages ± SD (n = 5) are illustrated).**

Adding N fertilizer could change plant $\delta^{15}$N by: (i) uptake of fertilizer-derived N with a different $\delta^{15}$N than the other soil N pools; generally, animal fertilizers with higher $\delta^{15}$N drive plant N isotopic compositions higher (Szpak et al., 2012); (ii) the impact of N fertilizer on soil internal N transformations and N loss reactions. These reactions discriminate against $^{15}$N (Hogberg, 1997) and greater N availability through addition of fertilizer could increase the $\delta^{15}$N of soil N pools available to plants (Szpak, 2014). The magnitude of the influence of animal N fertilization on plant $\delta^{15}$N, however, depends on the type of fertilizer (chemical composition, amount of N and its mineralization rate), the amount applied, and the manner and duration of application (Szpak, 2014). The significant increase in leaf $\delta^{15}$N of *P. glauca* from –1.3 ‰ in T0 to –0.9 ‰ in T2 and in

*E. macrourus* from 0.0 ‰ in T0 to +0.7 ‰ in T2 could be a consequence of these collective effects.

## Conclusion

In general, the grazing and fertilization experiments did not produce a clear pattern of change in the C and N isotopic composition of fine roots and leaves from *P. glauca* and *E. macrourus*. This outcome may be the result of three main factors: (i) the two main variables (grazing and fertilization) were considered separately, when in fact they are coupled in nature and work together as herbivory effects on plant communities, (ii) the short time period of the experiments (60 days) did not capture possible longer term isotopic effects of these variables, and (iii) the experiment employed too little fertilizer, and/or used insufficiently potent fertilizer (devolatilized, dried cow dung) and so it is possible that other growth chamber factors (T, RH or light intensity fluctuations) overshadowed the effect of fertilization and grazing variables in each experiment. The influence of grazing and natural fertilization on the N dynamics of Beringian-like grasslands should be studied farther in natural systems where all aspects of herbivory can be considered together.

**Appendix J: Accuracy and precision for standards associated with data presented in Chapter 4, exclusive of those used to generate calibration curves.**

| | | | $\delta^{13}$C (‰, VPDB) | | $\delta^{15}$N (‰, AIR) | | C (wt. %) | | N (wt. %) | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Standard** | **RUN ID** | **n** | **Mean** | ***SD*** | **Mean** | ***SD*** | **Mean** | ***SD*** | **Mean** | ***SD*** |
| **Keratin** | **CN 14-20** | **9** | −24.07 | 0.04 | | | | | | |
| | **CN 14-21** | **8** | −24.07 | 0.04 | | | | | | |
| | **CN 14-24** | **9** | −24.01 | 0.04 | | | | | | |
| | **CN 14-25** | **9** | −24.11 | 0.09 | | | | | | |
| | **CN 15-02**[a] | **10** | −24.11 | 0.03 | | | 48.03 | 0.77 | | |
| | **CN 15-03**[b] | **9** | −24.12 | 0.06 | | | | | | |
| | **CN 15-04** | **9** | −24.06 | 0.06 | | | 47.31 | 0.41 | | |
| | **CN 15-05** | **8** | −24.04 | 0.08 | | | 47.53 | 0.68 | | |
| | **CN 15-06**[a] | **11** | −24.10 | 0.05 | | | 48.20 | 0.46 | | |
| | **CN 15-07**[a] | **7** | −24.10 | 0.05 | | | 48.95 | 0.38 | | |
| | **EA alone 15-02** | **6** | | | | | 48.88 | 0.27 | | |
| | **EA alone 15-04** | **3** | | | | | 48.28 | 0.23 | | |
| | **NO 14-20** | **11** | | | +6.46 | 0.07 | | | 14.08 | 0.76 |
| | **NO 14-21** | **14** | | | +6.44 | 0.10 | | | 14.08 | 0.65 |
| | **NO 14-23** | **8** | | | +6.30 | 0.06 | | | 14.78 | 0.53 |
| | **NO 14-24** | **8** | | | +6.46 | 0.17 | | | 14.77 | 0.69 |
| | **NO 15-01** | **6** | | | +6.35 | 0.10 | | | 14.99 | 0.73 |
| | **NO 15-02** | **11** | | | +6.44 | 0.06 | | | 14.95 | 0.37 |
| | **NO 15-03** | **7** | | | +6.43 | 0.09 | | | 14.54 | 0.33 |
| | **NO 15-04** | **8** | | | +6.36 | 0.09 | | | 14.61 | 0.16 |
| | **NO 15-05** | **5** | | | +6.35 | 0.06 | | | 14.68 | 0.22 |
| | **NO 15-06 (Soil)** | **6** | | | +6.40 | 0.09 | | | 14.80 | 0.12 |
| | **NO 15-07 (Soil)** | **6** | | | +6.45 | 0.13 | | | 14.29 | 0.46 |
| | **NO 15-08** | **14** | | | +6.41 | 0.09 | | | 14.41 | 0.32 |
| **IAEA-CH-6** | **CN 14-20** | **3** | −10.47 | 0.03 | | | | | | |
| | **CN 14-21** | **2** | −10.48 | 0.07 | | | | | | |
| | **CN 14-24** | **3** | −10.53 | 0.07 | | | | | | |

| | | | $\delta^{13}$C (‰, VPDB) | | $\delta^{15}$N (‰, AIR) | | C (wt. %) | | N (wt. %) | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Standard** | **SESSION ID** | **n** | **Mean** | ***SD*** | **Mean** | ***SD*** | **Mean** | ***SD*** | **Mean** | ***SD*** |
| **IAEA-CH-6** | **CN 14-25** | **2** | −10.69 | 0.02 | | | | | | |
| | **CN 15-02** | **5** | −10.50 | 0.02 | | | | | | |
| | **CN 15-03** | **2** | −10.51 | 0.15 | | | | | | |
| | **CN 15-04** | **4** | −10.55 | 0.14 | | | | | | |
| | **CN 15-05** | **3** | −10.46 | 0.05 | | | | | | |
| | **CN 15-06** | **5** | −10.56 | 0.15 | | | | | | |
| | **CN 15-07** | **3** | −10.48 | 0.02 | | | | | | |
| **USGS40** | **CN 14-20** | **3** | | 0.06 | | | | | | |
| | **CN 14-21** | **3** | | 0.05 | | | | | | |
| | **CN 14-24** | **4** | | 0.03 | | | | | | |
| | **CN 14-25** | **4** | | 0.06 | | | | | | |
| | **CN 15-02** | **4** | | 0.08 | | | | | | |
| | **CN 15-03** | **4** | | 0.10 | | | | | | |
| | **CN 15-04** | **5** | | 0.03 | | | | | | |
| | **CN 15-05** | **3** | | 0.03 | | | | | | |
| | **CN 15-06** | **5** | | 0.03 | | | | | | |
| | **CN 15-07** | **3** | | 0.05 | | | | | | |
| | **NO 14-20** | **4** | | | | 0.11 | | | | |
| | **NO 14-21** | **5** | | | | 0.11 | | | | |
| | **NO 14-23** | **3** | | | | 0.05 | | | | |
| | **NO 14-24** | **3** | | | | 0.05 | | | | |
| | **NO 15-01** | **2** | | | | 0.01 | | | | |
| | **NO 15-02** | **3** | | | | 0.04 | | | | |
| | **NO 15-03** | **3** | | | | 0.07 | | | | |
| | **NO 15-04** | **4** | | | | 0.03 | | | | |
| | **NO 15-05** | **2** | | | | 0.10 | | | | |
| | **NO 15-06** | **2** | | | | 0.09 | | | | |
| | **NO 15-07 (Soil)** | **2** | | | | 0.04 | | | | |
| | **NO 15-08** | **5** | | | | 0.10 | | | | |
| **USGS41** | **CN 14-20** | **4** | | 0.11 | | | | | | |

| | | | $\delta^{13}$C (‰, VPDB) | | $\delta^{15}$N (‰, AIR) | | C (wt. %) | | N (wt. %) | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Standard** | **SESSION ID** | **n** | **Mean** | ***SD*** | **Mean** | ***SD*** | **Mean** | ***SD*** | **Mean** | ***SD*** |
| **USGS41** | **CN 14-21** | **3** | | 0.06 | | | | | | |
| | **CN 14-24** | **4** | | 0.11 | | | | | | |
| | **CN 14-25** | **4** | | 0.08 | | | | | | |
| | **CN 15-02** | **5** | | 0.04 | | | | | | |
| | **CN 15-03** | **4** | | 0.10 | | | | | | |
| | **CN 15-04** | **5** | | 0.26 | | | | | | |
| | **CN 15-05** | **4** | | 0.24 | | | | | | |
| | **CN 15-06** | **5** | | 0.15 | | | | | | |
| | **CN 15-07** | **3** | | 0.10 | | | | | | |
| | **NO 14-20** | **3** | | | | 0.19 | | | | |
| | **NO 14-21** | **4** | | | | 0.17 | | | | |
| | **NO 14-23** | **3** | | | | 0.07 | | | | |
| | **NO 14-24** | **2** | | | | 0.09 | | | | |
| | **NO 15-01** | **2** | | | | 0.15 | | | | |
| | **NO 15-02** | **6** | | | | 0.11 | | | | |
| | **NO 15-03** | **4** | | | | 0.24 | | | | |
| | **NO 15-04** | **4** | | | | 0.03 | | | | |
| | **NO 15-05** | **3** | | | | 0.19 | | | | |
| | **NO 15-06** | **3** | | | | 0.13 | | | | |
| | **NO 15-07 (Soil)** | **3** | | | | 0.11 | | | | |
| | **NO 15-08** | **7** | | | | 0.11 | | | | |
| **NIST 1547** | **NO 15-01** | **3** | | | | | | | 2.96 | 0.08 |
| | **NO 15-02** | **6** | | | | | | | 2.84 | 0.02 |
| | **NO 15-03** | **5** | | | | | | | 2.86 | 0.02 |
| | **NO 15-04** | **7** | | | | | | | 2.80 | 0.02 |
| | **NO 15-05** | **4** | | | | | | | 2.82 | 0.01 |
| | **NO 15-06** | **4** | | | | | | | 2.87 | 0.03 |
| | **NO 15-07 (soil)** | **4** | | | | | | | 2.80 | 0.05 |
| | **NO 15-08** | **10** | | | | | | | 2.79 | 0.02 |

| | | | $\delta^{13}$C (‰, VPDB) | | $\delta^{15}$N (‰, AIR) | | C (wt. %) | | N (wt. %) | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Standard** | **SESSION ID** | **n** | **Mean** | ***SD*** | **Mean** | ***SD*** | **Mean** | ***SD*** | **Mean** | ***SD*** |
| **Acetanilide** | [c]**1108 EA 15-02** | **11** | | | | | | 0.54 | | 0.12 |
| | **1108 EA 15-04** | **5** | | | | | | 0.89 | | |
| **High OC** | **1108 EA 15-01 (Soil)** | **5** | | | | | | | | 0.00 |
| **Low OC** | **1108 EA 15-01 (Soil)** | **4** | | | | | | | | 0.00 |
| **NBS-19** | **MPRP 15-01** | **2** | | 0.09 | | | | | | |
| **LSEVC** | **MPRP 15-01** | **2** | | 0.12 | | | | | | |

**[a] Elemental data were corrected, as described in Appendix B.**
**[b] The entire analytical session was repeated using the Fisons 1108 Elemental Analyzer for elemental composition.**
**[c] 1108 EA: Fisons 1108 Elemental Analyzer.**

**Appendix K: $\delta^{13}C$ (K.1) and $\delta^{15}N$ (K.2) of different plant parts and species examined for each $CO_2$ treatment.**

**Table K.1: $\delta^{13}C$ (‰, VPDB)**

| | | $\delta^{13}C$ (‰, VPDB) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | $CO_2$: Ambient | | | | | | | | |
| | | Chamber 1 | | | Chamber 2 | | | Chamber 3 | | |
| **Plant Species** | **Plant part** | **R1** | **R2** | **R3** | **R1** | **R2** | **R3** | **R1** | **R2** | **R3** |
| ***E. macrourus* (S)** | **L** | −30.4 | −29.4 | −30.8 | −30.9 | −29.9 | −31.2 | −30.8 | −30.9 | **−28.1** |
| | **RC** | −28.8 | −27.6 | −29.7 | −29.6 | −28.4 | **−30.2** | **−29.9** | −29.4 | −27.2 |
| | **FR** | −28.0 | −28.3 | −28.9 | −28.9 | **−28.5** | −28.5 | −28.4 | −28.9 | −28.3 |
| ***P. glauca* (S)** | **L** | −27.9 | −28.4 | −28.7 | - | - | −28.8 | - | - | −27.5 |
| | **RC** | −26.5 | −26.5 | −26.8 | - | - | −26.6 | - | - | −25.7 |
| | **FR** | −26.4 | −26.3 | −26.2 | - | - | −26.2 | - | - | −25.3 |
| ***B. pumpellianis* (S)** | **L** | −29.1 | −28.3 | −30.4 | −30.7 | −31.5 | −31.4 | −30.4 | −30.3 | −26.4 |
| | **RC** | −27.6 | −27.1 | −28.1 | −28.4 | −30.2 | −29.6 | −28.5 | **−28.8** | −24.8 |
| | **FR** | −27.8 | −27.8 | −28.1 | −29.5 | −29.8 | **−29.6** | −29.3 | **−29.0** | −26.3 |
| ***P. glauca* (RC)** | **L** | −30.7 | - | - | −30.2 | - | - | **−29.1** | - | - |
| | **RC** | −29.4 | - | - | −30.0 | - | - | **−27.9** | - | - |
| | **FR** | −29.7 | - | - | −29.2 | - | - | −26.8 | - | - |
| ***B. pumpellianis* (RC)** | **L** | −29.2 | - | - | −32.0 | - | - | −27.9 | - | - |
| | **RC** | −27.5 | - | - | −29.6 | - | - | −28.1 | - | - |
| | **FR** | −27.2 | - | - | −29.1 | - | - | −27.5 | - | - |
| ***F. altaica* (RC)** | **L** | −27.6 | - | - | −27.5 | - | - | −30.4 | - | - |
| | **RC** | −28.1 | - | - | −28.8 | - | - | −30.1 | - | - |
| | **FR** | −27.0 | - | - | −28.8 | - | - | −30.0 | - | - |
| **$CO_2$: 800 ppm** | | | | | | | | | | |
| ***E. macrourus* (S)** | **L** | **−30.4** | −30.8 | −30.8 | −33.7 | **−33.8** | −31.3 | −30.3 | −28.8 | −28.1 |
| | **RC** | −29.0 | −33.3 | −29.5 | −32.8 | −28.9 | −30.3 | −29.8 | −27.3 | −27.1 |
| | **FR** | −27.6 | −27.3 | −28.6 | −31.5 | −30.9 | −28.8 | −29.2 | −28.1 | **−28.2** |

**Table K.1. Cont'd.**

| | | Chamber 1 | | | Chamber 2 | | | Chamber 3 | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Plant Species** | **Plant part** | **R1** | **R2** | **R3** | **R1** | **R2** | **R3** | **R1** | **R2** | **R3** |
| ***P. glauca* (S)** | **L** | - | −29.4 | −30.3 | - | −31.1 | - | −30.2 | −28.6 | −30.4 |
| | **RC** | - | −29.2 | −29.2 | - | **−29.4** | - | −29.2 | −27.3 | −28.3 |
| | **FR** | - | −28.3 | **−28.1** | - | −28.1 | - | −28.3 | −26.9 | −28.3 |
| ***B. pumpellianis* (S)** | **L** | **−28.3** | −28.2 | −29.9 | −28.4 | −28.5 | −30.3 | −30.4 | −29.7 | −29.9 |
| | **RC** | −26.7 | −27.0 | **−28.9** | −26.9 | −27.3 | −29.1 | −29.3 | −28.2 | −29.3 |
| | **FR** | −27.6 | −27.8 | −29.2 | −27.8 | −28.2 | −29.2 | −29.7 | −28.4 | −29.1 |
| ***P. glauca* (RC)** | **L** | −31.8 | - | - | −31.8 | - | - | −31.8 | - | - |
| | **RC** | −30.4 | - | - | −30.5 | - | - | −31.0 | - | - |
| | **FR** | −28.5 | - | - | −30.3 | - | - | −31.0 | - | - |
| ***B. pumpellianis* (RC)** | **L** | - | - | - | −28.5 | - | - | −28.4 | - | - |
| | **RC** | - | - | - | −29.1 | - | - | −27.3 | - | - |
| | **FR** | - | - | - | −29.6 | - | - | −26.6 | - | - |
| ***F. altaica* (RC)** | **L** | −29.8 | - | - | −31.0 | - | - | −30.4 | - | - |
| | **RC** | −28.9 | - | - | −29.6 | - | - | −28.8 | - | - |
| | **FR** | −26.5 | - | - | −26.7 | - | - | −26.9 | - | - |
| | | **$CO_2$: 1000 ppm** | | | | | | | | |
| ***E. macrourus* (S)** | **L** | **−32.0** | −32.6 | −31.8 | −32.6 | −32.3 | −27.5 | −32.0 | −32.2 | −32.7 |
| | **RC** | −29.6 | −31.1 | −30.2 | −31.3 | −30.3 | - | −30.2 | −30.9 | −31.5 |
| | **FR** | −29.9 | −30.9 | −29.3 | −31.6 | −31.2 | −28.6 | −31.5 | −31.0 | −31.0 |
| ***P. glauca* (S)** | **L** | −29.0 | −31.0 | −31.6 | −32.8 | −29.8 | −30.7 | −31.5 | −31.5 | −29.9 |
| | **RC** | −28.8 | **−29.3** | −30.0 | −30.6 | **−28.8** | −29.0 | **−29.6** | −29.6 | −28.6 |
| | **FR** | **−28.5** | −29.3 | −29.9 | −30.5 | −29.0 | −29.3 | −29.2 | −29.2 | −29.3 |
| ***B. pumpellianis* (S)** | **L** | −30.1 | −28.6 | −29.6 | **−29.8** | −29.0 | −29.5 | −29.5 | −29.4 | −30.6 |
| | **RC** | −29.7 | −27.5 | −29.3 | −28.5 | −27.6 | −28.7 | −28.3 | −28.3 | −29.0 |
| | **FR** | −29.4 | −27.7 | −28.7 | −30.7 | −28.7 | −28.8 | −27.8 | −27.8 | −29.9 |
| ***Poa g* (RC)** | **L** | −29.9 | - | - | −31.2 | - | - | −29.6 | - | - |

**Table K.1. Cont'd.**

| | | Chamber 1 | | | Chamber 2 | | | Chamber 3 | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Plant Species** | **Plant part** | **R1** | **R2** | **R3** | **R1** | **R2** | **R3** | **R1** | **R2** | **R3** |
| ***P. glauca* (RC)** | **RC** | −31.2 | - | - | −30.8 | - | - | −32.3 | - | - |
| | **FR** | −33.2 | - | - | **−32.3** | - | - | −32.1 | - | - |
| ***B. pumpellianis* (RC)** | **L** | −29.2 | - | - | −27.2 | - | - | **−31.8** | - | - |
| | **RC** | −30.4 | - | - | −28.1 | - | - | −29.1 | - | - |
| | **FR** | −29.3 | - | - | −26.5 | - | - | −29.4 | - | - |

**(S): grown from seed**

**(RC): grown from root crown**

**R: Replicate**

**Boldface denotes average of duplicates.**

**No data are available for *F. altaica* at 1000 ppm $CO_2$ (T2).**

**Table K.2: $\delta^{15}$N (‰, AIR)**

| | | $\delta^{15}$N (‰, AIR) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | $CO_2$: Ambient | | | | | | | | |
| | | Chamber 1 | | | Chamber 2 | | | Chamber 3 | | |
| **Plant Species** | **Plant part** | **R1** | **R2** | **R3** | **R1** | **R2** | **R3** | **R1** | **R2** | **R3** |
| ***E. macrourus* (S)** | **L** | −1.2 | −0.3 | +2.0 | +1.8 | −0.9 | +0.5 | **+0.5** | +0.5 | −1.2 |
| | **RC** | −1.1 | −2.0 | +1.4 | +0.9 | **−1.1** | +0.4 | **−0.1** | +0.7 | −1.7 |
| | **FR** | −1.8 | −2.3 | −0.9 | +0.5 | **−1.9** | −0.2 | −1.2 | −0.9 | −3.9 |
| | **Soil** | +5.0 | +4.9 | +4.9 | +4.9 | +4.9 | **+5.2** | +5.1 | +5.6 | +5.0 |
| ***P. glauca* (S)** | **L** | 0.0 | −2.7 | −1.7 | - | - | −1.7 | - | - | −2.0 |
| | **RC** | +0.7 | −0.6 | −1.6 | - | - | −1.2 | - | - | −1.2 |
| | **FR** | −1.0 | −2.2 | −1.4 | - | - | 0.0 | - | - | −0.3 |
| | **Soil** | +4.6 | +4.9 | +4.8 | - | - | +4.6 | - | - | **+4.7** |
| ***B. pumpellianis* (S)** | **L** | +0.7 | +0.8 | −1.7 | +1.4 | +0.9 | +1.5 | +0.8 | +1.1 | +2.3 |
| | **RC** | −1.7 | −1.6 | −3.4 | −0.2 | −1.3 | +0.2 | −1.0 | **−0.7** | −0.5 |
| | **FR** | −0.3 | −1.6 | −2.1 | −0.1 | −1.2 | **+0.3** | −0.9 | **−0.7** | −0.9 |
| | **Soil** | +4.9 | +4.8 | +5.2 | +5.1 | +4.8 | +5.3 | +5.2 | +4.8 | +5.4 |
| ***P. glauca* (RC)** | **L** | +2.8 | - | - | +3.0 | - | - | **+1.2** | - | - |
| | **RC** | +1.8 | - | - | +1.1 | - | - | −3.0 | - | - |
| | **FR** | +1.9 | - | - | +1.5 | - | - | −1.1 | - | - |
| | **Soil** | +3.9 | - | - | +2.9 | - | - | **+2.0** | - | - |
| ***B. pumpellianis* (RC)** | **L** | +3.5 | - | - | +1.2 | - | - | +3.0 | - | - |
| | **RC** | −0.3 | - | - | −0.5 | - | - | +0.4 | - | - |
| | **FR** | +1.5 | - | - | +0.7 | - | - | +1.0 | - | - |
| | **Soil** | +4.4 | - | - | +4.6 | - | - | +4.9 | | |
| ***F. altaica* (RC)** | **L** | +0.4 | - | - | +2.3 | - | - | +2.3 | - | - |
| | **RC** | −2.1 | - | - | +2.3 | - | - | +0.4 | - | - |
| | **FR** | +0.7 | - | - | +1.4 | - | - | +1.6 | - | - |

**Table K.2. Cont'd.**

| | | Chamber 1 | | | Chamber 2 | | | Chamber 3 | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Plant Species** | **Plant part** | **R1** | **R2** | **R3** | **R1** | **R2** | **R3** | **R1** | **R2** | **R3** |
| | **Soil** | +2.5 | - | - | +5.3 | - | - | +5.1 | - | - |
| | | | | | **$CO_2$: 800 ppm** | | | | | |
| ***E. macrourus* (S)** | **L** | –2.6 | –1.3 | +0.8 | +5.3 | +1.3 | +1.5 | +3.9 | –2.4 | –2.1 |
| | **RC** | **–3.2** | –0.5 | 0.0 | +3.7 | +0.8 | **+0.4** | +2.9 | –3.8 | –2.8 |
| | **FR** | –0.3 | +1.4 | +0.9 | +0.8 | +0.1 | +1.6 | +2.8 | –1.3 | +1.7 |
| | **Soil** | +4.6 | +4.5 | +5.0 | **+4.2** | +4.5 | +4.5 | +4.3 | +4.4 | +5.3 |
| ***P. glauca* (S)** | **L** | +1.1 | +1.2 | +1.5 | - | +4.0 | - | +1.7 | **+0.4** | +4.9 |
| | **RC** | - | +1.0 | +1.3 | - | +2.3 | - | –0.1 | –1.8 | +3.8 |
| | **FR** | +2.4 | +0.8 | +2.1 | - | +3.2 | - | +1.3 | **+0.6** | +4.2 |
| | **Soil** | +3.7 | **+4.7** | +4.7 | - | **+4.4** | - | +5.0 | +4.3 | +4.7 |
| ***B. pumpellianis* (S)** | **L** | **–0.3** | +0.4 | +1.2 | +1.3 | +2.5 | +1.7 | +2.9 | +2.0 | +1.3 |
| | **RC** | –1.4 | –0.3 | –0.1 | –1.2 | +0.9 | –0.3 | +3.9 | +0.3 | –0.8 |
| | **FR** | –1.5 | –0.5 | **+1.3** | +0.8 | +1.4 | +0.2 | +1.5 | +1.6 | +1.8 |
| | **Soil** | +4.8 | +4.5 | +4.6 | +4.9 | +4.9 | +5.0 | +4.8 | +4.8 | +5.1 |
| ***P. glauca* (RC)** | **L** | +2.8 | - | - | +3.0 | - | - | +1.2 | - | - |
| | **RC** | +1.8 | - | - | +1.1 | - | - | –3.0 | - | - |
| | **FR** | +1.9 | - | - | +1.5 | - | - | –1.1 | - | - |
| | **Soil** | +3.9 | - | - | +2.9 | - | - | +2.0 | - | - |
| ***B. pumpellianis* (RC)** | **L** | +3.5 | - | - | +1.2 | - | - | +3.0 | - | - |
| | **RC** | –0.3 | - | - | –0.5 | - | - | +0.4 | - | - |
| | **FR** | +1.5 | - | - | +0.7 | - | - | **+1.0** | - | - |
| | **Soil** | +4.4 | - | - | +4.6 | - | - | +4.9 | - | - |
| ***F. altaica* (RC)** | **L** | +0.4 | - | - | +2.3 | - | - | **+2.3** | - | - |
| | **RC** | **–2.1** | - | - | +2.3 | - | - | +0.4 | - | - |
| | **FR** | +0.7 | - | - | +1.4 | - | - | +1.6 | - | - |
| | **Soil** | +2.5 | - | - | +5.3 | - | - | +5.1 | - | - |

**Table K.2. Cont'd.**

| | | Chamber 1 | | | Chamber 2 | | | Chamber 3 | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Plant Species** | **Plant part** | **R1** | **R2** | **R3** | **R1** | **R2** | **R3** | **R1** | **R2** | **R3** |
| | | | | | **$CO_2$: 1000 ppm** | | | | | |
| ***E. macrourus* (S)** | **L** | +4.2 | +3.4 | +4.8 | +4.4 | +3.7 | +4.1 | +4.5 | +4.7 | +4.1 |
| | **RC** | +3.8 | **+1.8** | +3.9 | +3.5 | +2.8 | +2.2 | +3.5 | **+1.1** | +3.0 |
| | **FR** | +2.8 | +1.6 | +2.5 | **+2.8** | +1.4 | +1.0 | **+2.8** | **+2.8** | +2.5 |
| | **Soil** | +4.7 | +4.8 | +4.7 | **+4.5** | +5.0 | +4.4 | +5.4 | +5.0 | +5.5 |
| ***P. glauca* (S)** | **L** | +0.9 | **−0.1** | +0.2 | - | +1.9 | −1.2 | +3.9 | +0.3 | −2.0 |
| | **RC** | −0.7 | −2.2 | −0.5 | - | −0.6 | −2.0 | +2.7 | −1.5 | −2.6 |
| | **FR** | −0.1 | −1.1 | −0.9 | - | −0.5 | −1.1 | +2.7 | −0.7 | −2.7 |
| | **Soil** | +5.1 | +4.9 | +4.7 | - | +5.0 | +5.7 | +5.3 | +5.0 | +4.6 |
| ***B. pumpellianis* (S)** | **L** | +5.1 | +3.8 | +5.2 | +4.0 | +4.1 | +5.6 | **+4.8** | +4.7 | +5.2 |
| | **RC** | +2.0 | +1.2 | +2.1 | +0.7 | +1.1 | +2.6 | +2.6 | +1.8 | +1.5 |
| | **FR** | +3.5 | +3.0 | +3.8 | **+2.0** | +3.3 | +4.4 | +4.1 | +3.6 | +4.3 |
| | **Soil** | +5.4 | **+5.1** | +4.9 | +4.8 | +4.9 | +4.6 | +4.5 | +4.8 | +4.8 |
| ***P. glauca* (RC)** | **L** | +0.4 | - | - | +1.3 | - | - | +2.9 | - | - |
| | **RC** | −2.8 | - | - | −3.1 | - | - | −1.8 | - | - |
| | **FR** | −1.7 | - | - | −1.2 | - | - | −0.7 | - | - |
| | **Soil** | +3.1 | - | - | +0.3 | - | - | **+1.2** | - | - |
| ***B. pumpellianis* (RC)** | **L** | +3.9 | - | - | +0.9 | - | - | +4.7 | - | - |
| | **RC** | 0.0 | - | - | **+1.8** | - | - | −1.0 | - | - |
| | **FR** | +1.5 | - | - | −0.5 | - | - | −0.5 | - | - |
| | **Soil** | +5.0 | - | - | +4.1 | - | - | +3.9 | - | - |

**(S): grown from seed; (RC): grown from root crown;**

**R: Replicate**

**Boldface denotes average of duplicates.**

**No data are available for *F. altaica* at 1000 ppm $CO_2$ (T2).**

**Appendix L: C (L.1) and N (L.2) contents of different plant parts and species examined in each treatment.**

**Table L.1: C (wt. %)**

| | | C (wt. %) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | $CO_2$: Ambient | | | | | | | | |
| | | Chamber 1 | | | Chamber 2 | | | Chamber 3 | | |
| **Plant Species** | **Plant part** | **R1** | **R2** | **R3** | **R1** | **R2** | **R3** | **R1** | **R2** | **R3** |
| ***E. macrourus* (S)** | **L** | 46.1 | 45.7 | 45.3 | 46.0 | 44.5 | **46.0** | 45.7 | 46.2 | 46.0 |
| | **RC** | 39.6 | 33.5 | 41.7 | 36.5 | 34.9 | **37.4** | **41.0** | 33.2 | 35.7 |
| | **FR** | 42.1 | 40.8 | 41.7 | 42.4 | **41.7** | 43.9 | 41.8 | 41.4 | 36.4 |
| ***P. glauca* (S)** | **L** | 46.8 | 45.8 | 46.0 | - | - | 44.9 | - | - | 44.5 |
| | **RC** | 40.1 | 33.6 | 37.1 | - | - | 40.0 | - | - | 33.5 |
| | **FR** | 42.1 | 39.7 | 40.2 | - | - | 43.7 | - | - | 36.8 |
| ***B. pumpellianis* (S)** | **L** | 44.5 | 44.1 | 44.1 | 44.6 | 44.7 | 45.5 | 45.5 | 45.6 | 46.0 |
| | **RC** | 39.8 | 41.3 | 39.0 | 40.2 | 40.1 | 39.0 | 41.4 | **40.2** | 39.6 |
| | **FR** | 37.6 | 33.5 | 27.7 | 41.9 | 42.9 | **37.4** | 39.3 | **40.3** | 43.1 |
| ***P. glauca* (RC)** | **L** | 44.9 | - | - | 45.0 | - | - | **42.1** | - | - |
| | **RC** | 45.3 | - | - | 45.2 | - | - | **39.2** | - | - |
| | **FR** | 44.6 | - | - | 45.1 | - | - | 38.9 | - | - |
| ***B. pumpellianis* (RC)** | **L** | 45.6 | - | - | 45.3 | - | - | 45.3 | - | - |
| | **RC** | 42.8 | - | - | 41.3 | - | - | 42.5 | - | - |
| | **FR** | 32.2 | - | - | 33.8 | - | - | 39.3 | - | - |
| ***F. altaica* (RC)** | **L** | 43.8 | - | - | 45.4 | - | - | 43.1 | - | - |
| | **RC** | 39.2 | - | - | 35.4 | - | - | 42.6 | - | - |
| | **FR** | 31.4 | - | - | 29.4 | - | - | 42.0 | - | - |
| **$CO_2$: 800 ppm** | | | | | | | | | | |
| ***E. macrourus* (S)** | **L** | **41.8** | 44.3 | 43.8 | 42.1 | **42.8** | 43.7 | 43.5 | 43.1 | **42.4** |
| | **FR** | 39.9 | 39.2 | 41.0 | 41.8 | 35.9 | 41.0 | 40.1 | 39.5 | **37.9** |
| | **RC** | 37.7 | 38.6 | 38.9 | 40.3 | 37.5 | 40.0 | 39.9 | 40.6 | 37.6 |

**Table L.1. Cont'd.**

| | | Chamber 1 | | | Chamber 2 | | | Chamber 3 | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Plant Species** | **Plant part** | **R1** | **R2** | **R3** | **R1** | **R2** | **R3** | **R1** | **R2** | **R3** |
| ***P. glauca* (S)** | **L** | 42.6 | 42.2 | 42.7 | - | 44.3 | - | 43.0 | 44.6 | 42.8 |
| | **RC** | - | 20.4 | 34.8 | - | **40.5** | - | 38.7 | 41.3 | 37.1 |
| | **FR** | 41.9 | 38.6 | **40.1** | - | 41.6 | - | 41.4 | 42.6 | 41.1 |
| ***B. pumpellianis* (S)** | **L** | 43.7 | 43.9 | 43.2 | 43.4 | 44.2 | 43.7 | 43.5 | 44.2 | 44.1 |
| | **RC** | 41.6 | 38.6 | **40.0** | 42.3 | 39.1 | 40.8 | 42.0 | 41.2 | 41.7 |
| | **FR** | **39.8** | 39.3 | 39.3 | 32.5 | 42.6 | 39.4 | 37.4 | 39.9 | 31.8 |
| ***P. glauca* (RC)** | **L** | 42.0 | - | - | 41.9 | - | - | 41.9 | - | - |
| | **RC** | 43.9 | - | - | 42.1 | - | - | 37.8 | - | - |
| | **FR** | 39.0 | - | - | 38.4 | - | - | 41.3 | - | - |
| ***B. pumpellianis* (RC)** | **L** | - | - | - | 42.8 | - | - | 44.9 | - | - |
| | **RC** | - | - | - | 38.1 | - | - | 41.5 | - | - |
| | **FR** | - | - | - | 41.5 | - | - | 25.3 | - | - |
| ***F. altaica* (RC)** | **L** | 43.6 | - | - | 42.7 | - | - | 43.1 | - | - |
| | **RC** | 43.0 | - | - | 40.3 | - | - | 40.4 | - | - |
| | **FR** | 43.1 | - | - | 40.4 | - | - | 26.3 | - | - |
| | | **$CO_2$: 1000 ppm** | | | | | | | | |
| ***E. macrourus* (S)** | **L** | **45.1** | 45.3 | 45.7 | **44.7** | 44.8 | 45.3 | 45.9 | 45.8 | 45.1 |
| | **RC** | 41.8 | 40.6 | 42.7 | 41.9 | 42.4 | 40.6 | 42.1 | 40.4 | 42.3 |
| | **FR** | 43.5 | 43.1 | 41.5 | 42.3 | 44.1 | 42.6 | 41.5 | 39.3 | 42.2 |
| ***P. glauca* (S)** | **L** | 39.6 | 45.7 | 44.9 | - | 45.9 | 44.0 | 46.1 | 45.5 | 44.4 |
| | **RC** | 41.3 | **39.9** | 41.7 | - | **42.5** | 39.2 | **37.4** | 38.8 | 39.0 |
| | **FR** | **43.0** | 41.2 | 43.5 | - | 42.1 | 40.3 | 43.4 | 41.4 | 40.6 |
| ***B. pumpellianis* (S)** | **L** | 45.1 | 45.4 | 45.5 | 46.1 | 46.2 | 45.9 | 46.0 | 45.4 | 45.8 |
| | **RC** | 44.0 | 42.5 | 44.3 | 44.0 | 41.8 | 43.7 | 41.3 | 42.8 | 43.1 |
| | **FR** | 41.9 | 39.6 | 43.1 | 40.7 | 39.6 | 43.5 | 42.6 | 40.4 | 43.4 |
| ***P. glauca* (RC)** | **L** | 44.8 | - | - | **44.7** | - | - | 44.4 | - | - |

**Table L.1. Cont'd.**

| | | Chamber 1 | | | Chamber 2 | | | Chamber 3 | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Plant Species** | **Plant part** | **R1** | **R2** | **R3** | **R1** | **R2** | **R3** | **R1** | **R2** | **R3** |
| ***P. glauca* (RC)** | **RC** | 40.7 | - | - | 41.0 | - | - | 41.7 | - | - |
| | **FR** | 39.0 | - | - | 41.2 | - | - | 43.2 | - | - |
| ***B. pumpellianis* (RC)** | **L** | 46.9 | - | - | 45.9 | - | - | **45.8** | - | - |
| | **RC** | 43.4 | - | - | 42.0 | - | - | 40.5 | - | - |
| | **FR** | 41.8 | - | - | 41.4 | - | - | 41.7 | - | - |

**(S): grown from seed**

**(RC): grown from root crown**

**R: Replicate**

**Boldface denotes average of duplicates.**

**No data are available for *F. altaica* at 1000 ppm $CO_2$ (T2).**

**Table L.2: N (wt. %)**

| | | N (wt. %) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | $CO_2$: Ambient | | | | | | | | |
| | | Chamber 1 | | | Chamber 2 | | | Chamber 3 | | |
| **Plant Species** | **Plant part** | **R1** | **R2** | **R3** | **R1** | **R2** | **R3** | **R1** | **R2** | **R3** |
| ***E. macrourus* (S)** | **L** | 2.8 | 2.7 | 2.5 | 2.6 | 2.6 | 2.6 | **2.7** | 2.8 | 2.7 |
| | **RC** | 2.5 | 2.1 | 2.6 | 2.1 | 2.2 | **2.2** | **2.2** | 1.8 | 1.9 |
| | **FR** | 2.1 | 2.1 | 1.6 | 2.1 | **2.3** | 2.2 | 2.0 | 2.1 | 1.9 |
| | **Soil** | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | **0.1** | 0.1 | 0.1 | 0.1 |
| ***P. glauca* (S)** | **L** | 3.2 | 3.6 | 3.2 | - | - | 3.1 | - | - | 2.8 |
| | **RC** | 2.2 | 1.7 | 2.6 | - | - | 2.7 | - | - | 2.8 |
| | **FR** | 2.0 | 2.0 | 1.9 | - | - | 2.0 | - | - | 1.9 |
| | **Soil** | 0.1 | 0.1 | 0.1 | - | - | 0.1 | - | - | **0.2** |
| ***B. pumpellianis* (S)** | **L** | 2.1 | 2.8 | 2.4 | 2.6 | 2.7 | 2.5 | 2.8 | 2.5 | 2.3 |
| | **RC** | 2.3 | 3.3 | 2.5 | 2.3 | 2.6 | 2.0 | 2.5 | **2.2** | 2.8 |
| | **FR** | 1.4 | 1.3 | 1.1 | 1.6 | 1.9 | **1.3** | 1.6 | **1.6** | 1.4 |
| | **Soil** | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| ***P. glauca* (RC)** | **L** | 1.3 | - | - | 1.4 | - | - | **2.3** | - | - |
| | **RC** | 1.0 | - | - | 0.8 | - | - | 1.5 | - | - |
| | **FR** | 0.8 | - | - | 0.8 | - | - | 1.4 | - | - |
| | **Soil** | 0.2 | - | - | 0.2 | - | - | **0.4** | - | - |
| ***B. pumpellianis* (RC)** | **L** | 2.7 | - | - | 3.3 | - | - | 2.9 | - | - |
| | **RC** | 2.4 | - | - | 2.7 | - | - | 2.6 | - | - |
| | **FR** | 1.2 | - | - | 1.2 | - | - | 1.4 | - | - |
| | **Soil** | 0.1 | - | - | 0.1 | - | - | 0.1 | | |
| ***F. altaica* (RC)** | **L** | 1.6 | - | - | 1.4 | - | - | 2.2 | - | - |
| | **RC** | 1.2 | - | - | 1.0 | - | - | 2.4 | - | - |
| | **FR** | 0.8 | - | - | 0.8 | - | - | 1.2 | - | - |

**Table L.2. Cont'd.**

| | | Chamber 1 | | | Chamber 2 | | | Chamber 3 | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Plant Species** | **Plant part** | **R1** | **R2** | **R3** | **R1** | **R2** | **R3** | **R1** | **R2** | **R3** |
| | **Soil** | 0.3 | - | - | 0.3 | - | - | 0.3 | - | - |
| | | | | | **$CO_2$: 800 ppm** | | | | | |
| ***E. macrourus* (S)** | **L** | 2.6 | 2.4 | 1.9 | 1.7 | 2.2 | 1.8 | 1.5 | 2.2 | 2.3 |
| | **RC** | **3.1** | 2.0 | 1.7 | 1.1 | 1.8 | **2.0** | 1.1 | 2.2 | 2.8 |
| | **FR** | 2.4 | 2.2 | 2.1 | 1.4 | 1.8 | 2.1 | 1.5 | 2.0 | 1.8 |
| | **Soil** | 0.2 | 0.1 | 0.1 | 0.1 | 0.2 | 0.1 | 0.1 | 0.1 | 0.1 |
| ***P. glauca* (S)** | **L** | 2.8 | 2.8 | 2.6 | - | 2.3 | - | 2.3 | **2.5** | 2.2 |
| | **RC** | - | 1.8 | 1.6 | - | 1.8 | - | 1.8 | 2.1 | 1.6 |
| | **FR** | 2.1 | 1.8 | 2.1 | - | 1.6 | - | 1.8 | **1.9** | 1.3 |
| | **Soil** | 0.3 | 0.2 | 0.1 | - | 0.2 | - | 0.1 | 0.2 | 0.1 |
| ***B. pumpellianis* (S)** | **L** | **2.5** | 2.2 | 2.3 | 2.1 | 1.9 | 2.6 | 2.3 | 2.2 | 2.4 |
| | **RC** | 2.7 | 2.1 | 2.1 | 2.1 | 1.2 | 2.2 | 2.5 | 1.7 | 2.1 |
| | **FR** | 1.3 | 1.4 | **1.3** | 1.1 | 1.3 | 1.4 | 1.2 | 1.3 | 1.2 |
| | **Soil** | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| ***P. glauca* (RC)** | **L** | 3.4 | - | - | 1.6 | - | - | 1.9 | - | - |
| | **RC** | 1.4 | - | - | 1.7 | - | - | 1.4 | - | - |
| | **FR** | 0.9 | - | - | 1.2 | - | - | 0.8 | - | - |
| | **Soil** | 0.4 | - | - | 0.2 | - | - | 0.5 | - | - |
| ***B. pumpellianis* (RC)** | **L** | - | - | - | 1.7 | - | - | 2.7 | - | - |
| | **RC** | - | - | - | 2.3 | - | - | 2.4 | - | - |
| | **FR** | - | - | - | 1.3 | - | - | **0.9** | - | - |
| | **Soil** | - | - | - | 0.6 | - | - | 0.2 | - | - |
| ***F. altaica* (RC)** | **L** | 1.7 | - | - | 2.0 | - | - | **1.8** | - | - |
| | **RC** | **1.2** | - | - | 1.3 | - | - | 1.3 | - | - |
| | **FR** | 0.8 | - | - | 1.3 | - | - | 0.9 | - | - |
| | **Soil** | 0.3 | - | - | 0.2 | - | - | 0.4 | - | - |

**Table L.2. Cont'd.**

| | | Chamber 1 | | | Chamber 2 | | | Chamber 3 | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Plant Species** | **Plant part** | **R1** | **R2** | **R3** | **R1** | **R2** | **R3** | **R1** | **R2** | **R3** |
| | | | | | **$CO_2$: 1000 ppm** | | | | | |
| ***E. macrourus* (S)** | **L** | 1.9 | 1.6 | 2.1 | 1.3 | 1.8 | 1.6 | 1.4 | 1.2 | 1.3 |
| | **RC** | 2.2 | **1.8** | 2.0 | 1.4 | 2.2 | 2.3 | 1.5 | **1.1** | 1.1 |
| | **FR** | 2.0 | 1.9 | 1.9 | **1.4** | 1.8 | 2.1 | **1.3** | **1.2** | 1.4 |
| | **Soil** | 0.1 | 0.1 | 0.1 | **0.1** | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| ***P. glauca* (S)** | **L** | 2.9 | **2.9** | 2.7 | - | 2.6 | 3.1 | 2.5 | 3.4 | 3.6 |
| | **RC** | 2.5 | 2.7 | 2.5 | - | 2.4 | 3.0 | 1.8 | 2.0 | 2.6 |
| | **FR** | 1.9 | 2.0 | 2.2 | - | 1.8 | 2.0 | 1.5 | 1.8 | 1.9 |
| | **Soil** | 0.1 | 0.1 | 0.1 | - | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| ***B. pumpellianis* (S)** | **L** | 1.7 | 2.1 | 1.7 | 2.2 | 1.7 | 1.4 | **1.6** | 1.7 | 1.9 |
| | **RC** | 1.9 | 2.2 | 1.7 | 2.0 | 2.1 | 1.6 | 1.5 | 1.8 | 1.9 |
| | **FR** | 1.1 | 1.2 | 1.1 | **1.3** | 1.3 | 1.1 | 1.0 | 1.0 | 1.0 |
| | **Soil** | 0.1 | **0.1** | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| ***P. glauca* (RC)** | **L** | 3.1 | - | - | 2.5 | - | - | 2.9 | - | - |
| | **RC** | 2.0 | - | - | 1.7 | - | - | 1.8 | - | - |
| | **FR** | 1.4 | - | - | 1.4 | - | - | 1.4 | - | - |
| | **Soil** | 0.3 | - | - | 0.6 | - | - | **0.4** | - | - |
| ***B. pumpellianis* (RC)** | **L** | 2.4 | - | - | 2.0 | - | - | 3.0 | - | - |
| | **RC** | 2.4 | - | - | **1.8** | - | - | 1.8 | - | - |
| | **FR** | 1.1 | - | - | 1.2 | - | - | 1.6 | - | - |
| | **Soil** | 0.1 | - | - | 0.1 | - | - | 0.1 | | |

**(S): grown from seed; (RC): grown from root crown.**

**R: Replicate**

**Boldface denotes average of duplicates.**

**No data are available for *F. altaica* at 1000 ppm $CO_2$ (T2).**

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2014-2015

Western University Queen Elizabeth II Scholarship (QEII)
2013-2014

Graduate Thesis Research Assistant Award (GTRA)
2012-2013

**Related Work Experience**

Teaching Assistant
The University of Western Ontario
2011-2013